

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
9 October 2003 (09.10.2003)

PCT

(10) International Publication Number
WO 03/082898 A2

- (51) International Patent Classification⁷: C07K (74) Agents: FORSTMAYER, Dietmar et al.; Boeters & Bauer, Bereiteranger 15, 81541 München (DE).
- (21) International Application Number: PCT/EP03/03307
- (22) International Filing Date: 28 March 2003 (28.03.2003) (81) Designated States (*national*): AB, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/368,197 28 March 2002 (28.03.2002) US
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- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— *without international search report and to be republished upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 03/082898 A2

(54) Title: NOVEL ANALOGUES OF GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE

(57) Abstract: The present invention relates to novel C-terminal truncated fragments and novel N-terminal modified analogues of gastric inhibitory polypeptide as well as various GIP analogues with a reduced peptide bond or alterations of the amino acids close to the dipeptidyl peptidase IV (DPIV)-specific cleavage site providing improved DPIV-resistance and prolonged half-life. Further the invention relates to novel analogs with different linkers between potential receptors binding sites of GIP. The compounds of the present invention and their pharmaceutically acceptable salts are useful in treating GIP-receptor mediated conditions, such as non-insulin dependent diabetes mellitus and obesity.

Novel Analogues of Glucose-dependent Insulinotropic Polypeptide

Field of the invention

- 5 The present invention relates to the area of novel analogues of Glucose-dependent Insulinotropic Polypeptide (GIP), pharmaceutical compositions containing said compounds, and the use of said compounds as GIP-receptor agonists or antagonists for the treatment of GIP-receptor mediated conditions.

10

Background Art

- The incretin GIP (glucose-dependent insulinotropic polypeptide), a 42 amino acid peptide, is released from the K-cells of the small intestine into the blood in response to oral nutrient ingestion. GIP inhibits the secretion of gastric acid and promotes the release of insulin from pancreatic islet cells [1,2]. It has been shown that the combined effects of GIP and glucagon-like peptide-1₇₋₃₆ (tGLP-1) are sufficient to explain the full incretin effect of the entero-insular axis [3]. GIP and the related hormone, tGLP-1, have been considered to be involved in the pathogenesis of type II (non-insulin dependent) diabetes mellitus. The physiological actions of the incretins, and especially of GLP-1, are not only manifested by enhanced insulin secretion but also by inhibition of gastric emptying [4] and suppression of glucagon release [5,6,7,8] and may result in an improved glucose tolerance. Additionally, GIP is an important regulator of adipocyte function and changes in GIP function may contribute to progression of obesity in man [9].

- In serum, both incretins, GIP and tGLP-1, are degraded by dipeptidyl peptidase IV (DPIV). The resulting short biological half-life (~2 min *in vivo*) limits the therapeutic use of GIP and tGLP-1 [10,11,12]. In the case of tGLP-1, several studies have been directed at obtaining biologically active tGLP-1 analogues with improved DPIV-resistance [13,14]. For GIP, a preliminary study was performed to obtain analogues with improved DP IV-resistance [20]. Recently it

was demonstrated that the full-length GIP (1-30) analogs: Tyr¹-glucitol-GIP [15] and (Pro³)GIP [20, 21] display DP IV-resistance and enhanced bioactivity.

The GIP-receptor, a member of the G-protein-coupled receptor family [16,17],
5 has a high specificity for GIP and does not bind other peptides of the glucagon family. For this reason, GLP-1/GIP chimeric peptides show nearly no affinity for the GIP-receptor [18]. From such studies it has been concluded that the GIP₁₋₃₀ sequence of the GIP₁₋₄₂ molecule is crucial for receptor recognition. This was confirmed by Gelling et al [19] who showed that GIP₆₋₃₀-amide (GIP_{6-30a})
10 contains the high affinity binding region of GIP₁₋₄₂ but exhibits antagonist activity, as do other N-terminally truncated forms.

The following patent applications have been filed related to the effects of GIP analogues on the function of various target organs and their potential use as
15 therapeutic agents :

DE 199 21 537 discloses a method for extending the survival of insulin producing β -cells by stimulation of their proliferation and prevention of their programmed cell death. The specific goal is to increase the endogenous insulin
20 content and insulin response to elevated blood glucose levels. An important component of this invention is the activation of protein kinase B/Akt in insulin producing β -cells in response to the administration of effectors such as GLP-1, GIP, Exendin-4 or GLP-1 receptor agonists or GIP-receptor agonists.

25 EP 0479 210 discloses a novel GIP analogue of the formula GIP(1-13)-X-GIP(15-30)-Y, wherein X is an amino acid residue other than Met, and Y is selected from homoserine (inclusive homoserine-lactone) and shall be referred to as "Hse", homoserine amide (Hse-NH₂), H-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln-Hse or H-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-
30 Gln-Hse-NH₂.

WO 98/24464 discloses an antagonist of glucose-dependent insulinotropic polypeptide (GIP) consisting essentially of a 24 amino acid polypeptide corresponding to positions 7-30 of the sequence of GIP, a method of treating non-insulin dependent diabetes mellitus and a method of improving glucose tolerance in a non-insulin dependent diabetes mellitus patient.

WO 00/58360 discloses peptides, which stimulate the release of insulin. This invention especially provides a process of N terminally-modifying GIP and the use of the peptide analogues for treatment of diabetes. The specific peptide analog, which is disclosed in this invention, comprises at least 15 amino acid residues from the N terminal end of GIP (1-42). In another embodiment, Tyr¹ glucitol GIP (1-42) is disclosed.

WO 00/20592 discloses GIP or anti-idiotypic antibodies of GIP or fragments thereof as GIP-analogs for maintaining or increasing bone density or bone formation.

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- 35

Summary of the Invention

The present invention relates to novel C-terminally truncated fragments and novel N-terminally modified analogues of gastric inhibitory polypeptide as well as various GIP analogues with a reduced peptide bond or alterations of the amino acids close to the dipeptidyl peptidase IV (DPIV) specific cleavage site with the aim of improved DPIV-resistance and prolonging half-life. Further the invention relates to novel analogues with different linkers between potential receptor binding sites of GIP.

The compounds of the present invention and their pharmaceutically acceptable salts are useful in treating conditions in which GIP-receptor function may be altered, including non-insulin dependent diabetes mellitus and obesity. Two specific applications are proposed:

1. The compounds of the present invention are able to potentiate glucose-dependent proliferation of pancreatic β -cells.
2. The compounds of the present invention have anti-apoptotic effects on pancreatic β -cells.

Brief description of the Drawings

Figure 1: Cyclic AMP production by N-terminally modified GIP analogues in CHO-K1 cells stably transfected with the rat pancreatic islet GIP-receptor (wtGIPR cells). Stimulation was allowed to occur for 30 minutes at 37°C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA and 0.5 mM IBMX, with or without peptides at the concentrations shown. Cell contents were extracted in ice-cold 70% ethanol, dried in vacuo, and cyclic AMP measured by radioimmunoassay. Data represent the mean \pm SEM of at least three independent experiments. Data are normalized to the maximal cAMP stimulated by GIP_{1-30NH₂}.

Figure 2: Cyclic AMP production in wtGIPR cells by modified GIP1-14OH peptides, relative to native hormone. Stimulation was allowed to occur for 30 minutes at 37C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA and 0.5 mM IBMX, with or without peptides at the concentrations shown. Cell contents were extracted in ice-cold 70% ethanol, dried in vacuo, and cyclic AMP measured by radioimmunoassay. Data represent the mean \pm SEM of at least three independent experiments. Data are normalized to cell number.

Figure 3: Cyclic AMP production by modified GIP1-14OH peptides (20 micromolar) in wtGIPR cells. Data are from Fig. 2, represented as a factor of the basal cyclic AMP content in the cells. * = $P < 0.05$ versus 1 nM stimulated cAMP by GIP1-42; # = $P < 0.05$ versus basal cyclic AMP ($n \geq 3$).

Figure 4: Cyclic AMP production by GIP1-14OH peptides (40 micromolar) modified by alanine scanning. At positions 2 and 13, where alanines reside in the native primary sequence, the amino acids in those positions were replaced with those found in the primary sequence of the related hormone, glucagon. Stimulation was allowed to occur for 30 minutes at 37C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA and 0.5 mM IBMX, with or without peptides at the concentrations shown. Cell contents were extracted in ice-cold 70% ethanol, dried in vacuo, and cyclic AMP measured by radioimmunoassay. Data are represented as a factor of the basal cyclic AMP content in the cells. * = $P < 0.05$ versus 1 nM stimulated cAMP by GIP1-42; # = $P < 0.05$ versus basal cyclic AMP ($n \geq 3$).

Figure 5: Cyclic AMP production in wtGIPR cells by modified GIP peptides having core sequence deletions or alpha-helical insertions, relative to native hormone. Stimulation was allowed to occur for 30 minutes at 37C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA and 0.5 mM IBMX, with or without peptides at the concentrations shown. Cell contents were extracted in ice-cold 70% ethanol, dried in vacuo, and cyclic AMP measured by radioimmunoassay.

Data represent the mean \pm SEM of at least three independent experiments. Data are normalized to cell number.

Figure 6: Cyclic AMP production in wtGIPR cells by modified GIP peptides having core sequence deletions or alpha-helical insertions, relative to native hormone. Stimulation was allowed to occur for 30 minutes at 37C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA and 0.5 mM IBMX, with or without peptides at the concentrations shown. Cell contents were extracted in ice-cold 70% ethanol, dried in vacuo, and cyclic AMP measured by radiolimmunoassay. Data represent the mean \pm SEM of at least three independent experiments. Data are normalized to cell number.

Figure 7: Cyclic AMP production in wtGIPR cells by modified GIP peptides having N-terminal modifications or cyclicized between amino acids 16 and 21, relative to native hormone. Stimulation was allowed to occur for 30 minutes at 37C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA and 0.5 mM IBMX, with or without peptides at the concentrations shown. Cell contents were extracted in ice-cold 70% ethanol, dried in vacuo, and cyclic AMP measured by radioimmunoassay. Data represent the mean \pm SEM of at least three independent experiments. Data are normalized to the maximal cAMP produced by GIP1-42OH.

Figure 8: Competitive binding inhibition studies on Intact wtGIPR cells using 125 I-GIP versus modified GIP1-14 peptides at the concentrations shown. Equilibrium binding was achieved following 12-16 hour incubation at 4C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA + 1% Trasylol (aprotinin). Unbound label was removed during washing steps, and cells were solubilized in 0.2 M NaOH and transferred to borosilicate tubes for counting cell associated radioactivity. Non-specific binding was defined as cell associated radioactivity detected in the presence of 1 micromolar GIP1-42. Data represent the mean \pm SEM of greater than 3 experiments, and are normalized to the specific binding of 125 I-GIP measured in the absence of competitor (Bo).

Figure 9: Percent displacement of ^{125}I -GIP from wtGIPR cells by 50 micromolar peptide analogues (GIP1-14 peptides with alanine, serine, tyrosine, D-alanine, D-proline, reduced P2-P3 peptide bond, or BTD substitutions/modifications).

5 Equilibrium binding was achieved following 12-16 hour incubation at 4C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA + 1% Trasylol (aprotinin). Unbound label was removed during washing steps, and cells were solubilized in 0.2 M NaOH and transferred to borosilicate tubes for counting cell associated radioactivity. Non-specific binding was defined as cell associated
10 radioactivity detected in the presence of 1 micromolar GIP1-42. Data represent the mean \pm SEM of greater than 3 experiments. * = $P < 0.05$ versus % displacement by GIP1-14; # = $P < 0.05$ versus zero displacement (i.e. only A3 and A5 were unable to displace measurable ^{125}I -GIP binding).

15 Figure 10: Competitive binding inhibition studies on intact wtGIPR cells using ^{125}I -GIP versus GIP peptides having core sequence deletions or alpha-helical insertions, relative to native hormone at the concentrations shown. Equilibrium
binding was achieved following 12-16 hour incubation at 4C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA + 1% Trasylol (aprotinin). Unbound
20 label was removed during washing steps, and cells were solubilized in 0.2 M NaOH and transferred to borosilicate tubes for counting cell associated radioactivity. Non-specific binding was defined as cell associated radioactivity detected in the presence of 1 micromolar GIP1-42. Data represent the mean \pm SEM of greater than 3 experiments, and are normalized to the specific binding
25 of ^{125}I -GIP measured in the absence of competitor (B_0).

Figure 11: Competitive binding inhibition studies on intact wtGIPR cells using ^{125}I -GIP versus GIP peptides having core sequence deletions or alpha-helical insertions, relative to native hormone at the concentrations shown. Equilibrium
30 binding was achieved following 12-16 hour incubation at 4C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 + 0.1% BSA + 1% Trasylol (aprotinin). Unbound label was removed during washing steps, and cells were solubilized in 0.2 M

NaOH and transferred to borosilicate tubes for counting cell associated radioactivity. Non-specific binding was defined as cell associated radioactivity detected in the presence of 1 micromolar GIP1-42. Data represent the mean \pm SEM of greater than 3 experiments, and are normalized to the specific binding of ^{125}I -GIP measured in the absence of competitor (Bo).

Figure 12: Intraperitoneal glucose tolerance test in anaesthetized (65 mg/Kg sodium pentobarbital IP) male Wistar rats with synthetic GIP analogues. Intravenous (jugular) infusion of saline or peptide (A: 1 pmol/min/100 g body weight or B: 100 pmol/min/100 g body weight) was started 5 minutes prior to 1 g glucose/Kg body weight intraperitoneal injection. Blood samples were taken from the tail vein prior to infusion (basal sample) and at 10 minute intervals for one hour. Blood glucose measurements were made using hand-held glucometers. * = $P < 0.05$ versus saline control. Data represent the mean \pm SEM of ≥ 4 animals.

Figure 13: Oral glucose tolerance test (1 g/Kg BW) in conscious unrestrained male Wistar rats with or without subcutaneous peptide injection (8 nmol/Kg BW in 500 μL volume; or 80 nmol/Kg BW in one case). Basal samples were obtained from the tail vein prior to oral glucose and peptide injection. Samples were then obtained at the indicated time points to measure whole blood glucose using a hand held glucometer. Data represent the mean \pm SEM of ≥ 4 animals.

Figure 14: Integrated glucose responses from conscious unrestrained male Wistar rats having concurrent oral glucose tolerance test and subcutaneous peptide injections (i.e. integrated data from figure 13). Area under the curve was calculated using the trapezoidal method with baseline subtraction. Data represent the mean \pm SEM of ≥ 4 animals.

Figure 15: GIP potentiates 11 mM glucose induced cell growth to a similar level as GH (A) and GLP-1 (B) in INS-1 (832/13) cells. Cells were serum starved before and during the course of the experiment. Final cell numbers were

always greater than initial plating densities, indicative of mitogenesis, and final cell numbers were quantified fluorometrically by CYQUANT™. Values are means of 5 (A) and 4 (B) individual experiments done in triplicate, where * represents $p < 0.05$.

5

Figure 16: GIP promotes INS-1 (832/13) cell survival during glucose deprivation in a concentration-dependent manner. Cells were serum and glucose starved for 48 h, and GIP was added for the final 24 h period of culture. Final cell numbers were always less than initial plating density, indicating cell death was occurring, and final cell numbers were quantified fluorometrically by CYQUANT™. Values are means of 3 (A) and 4 (B) individual experiments done in triplicate, where * represents $p < 0.05$.

Figure 17: GIP promotion of INS-1 (832/13) cell survival during glucose deprivation involves p38 MAPK. Protein kinase inhibitors were added to the medium 15 min. prior to the final 24 h culture in the absence or presence of 100 nM GIP. The PKA inhibitor, H89, was unable to reverse GIP (A) or Forskolin (B) mediated cell survival. Wortmannin has deleterious effects on cell survival (C), which were partially reversed by GIP. Panel D represents the involvement of p38 MAP kinase, via specific inhibition with SB202190. Final cell numbers were quantified fluorometrically by CYQUANT™, and data represent means of 3-8 experiments done in triplicate, where * and # represent $p < 0.05$ vs. respective controls.

Figure 18: GIP ablates 0 mM glucose (A) and STZ (B) induced caspase-3 activity in INS-1 (832/13) cells. Cells were serum starved before and during the experiment, and 100 nM GIP, 10 μ M forskolin, or 100 nM GLP-1 were added for 6 h in the presence and absence of glucose (3 mM) or STZ to assess effects on caspase-3 activity. Caspase-3 activity was quantified using the aminomethylcoumarin (AMC)-derived substrate, Z-DEVD-AMC, and correcting for total protein concentration, where * and # represent $p < 0.05$ vs. respective

controls (A, n=3; B, n= 5). Relative activity was ensured to be specific by using the caspase-3 inhibitor Ac-DEVD-CHO (A, inset).

5 Detailed description of the invention

The present invention relates to novel C-terminally truncated fragments and novel N-terminally modified analogues of Glucose-dependent Insulinotropic Polypeptide as well as various GIP analogues with a reduced peptide bond or
10 alterations of the amino acids close to the dipeptidyl peptidase IV (DPIV) specific cleavage site with the aim of improving DPIV-resistance and a prolonging half-life. The amino acid alterations according to the present invention include residues of L-amino acids, D-amino acids, proteinogenic and non-proteinogenic amino acids. Proteinogenic amino acids are defined as
15 natural protein-derived α -amino acids. Non-proteinogenic amino acids are defined as all other amino acids, which are not building blocks of common natural proteins.

Further, the invention relates to novel analogues with different linkers between potential receptor binding sites of GIP.

20

More particularly, the present invention relates to novel GIP analogues with the general amino acid sequence shown in formula (1):

25 Tyr-A-B-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met (1)

wherein A and B are amino acid residues including D-amino acid residues, N-methylated amino acid residues and any other non-proteinogenic amino acid
30 residues. Additionally, the N-terminus of the tyrosine residue in position 1 can be modified by alkylation, sulphonylation, glycation, homoserine formation, pyroglutamic acid formation, disulphide bond formation, deamidation of asparagine or glutamine residues, methylation, t-butylation, t-

butyloxycarbonylation, 4-methylbenzylation, thioanysilation, thiocresylation, benzyloxymethylation, 4-nitrophenylation, benzyloxycarbonylation, 2-nitrobenzoylation, 2-nitrosulphenylation, 4-toluenesulphonylation, pentafluorophenylation, diphenylmethylation, 2-chlorobenzyloxycarbonylation, 5 2,4,5-trichlorophenylation, 2-bromobenzyloxycarbonylation, 9-fluorenylmethyloxycarbonylation, triphenylmethylation, 2,2,5,7,8-pentamethylchroman-6-sulphonylation, hydroxylation, oxidation of methionine, formylation, acetylation, anisylation, benzylation, benzoylation, trifluoroacetylation, carboxylation of aspartic acid or glutamic acid, 10 phosphorylation, sulphation, cysteinylolation, glycolysation with pentoses, deoxyhexoses, hexosamines, hexoses or N-acetylhexosamines, farnesylation, myristoylation, blotinylation, palmitoylation, stearoylation, geranylgeranylation, glutathionylation, 5'-adenosylation, ADP-ribosylation, modification with N-glycolylneuraminic acid, N-acetylneuraminic acid, pyridoxal phosphate, lipoic 15 acid, 4'-phosphopantetheine, and N-hydroxysuccinimide. The peptide of formula 1 can be modified by the introduction of at least one ϵ -amino fatty acid acylated lysine in any amino acid position.

The sequence of native GIP (1-14) is excluded from the present invention.

20 The most preferred compounds of formula (1) are D-Ala²-GIP (1-14), Pro³-GIP (1-14) and Ser²-GIP (1-14).

In another preferred embodiment the present invention relates to GIP analogues with a reduced peptide bond, shown by formula (2) of

25

Tyr-Ala- Ψ (CH₂NH₂)-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met
(2a)

30 Tyr-Ala- ψ (CH₂NH)-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-
Ile-His- Gln-Gln-Asp-
Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys (2b)

In a further embodiment, the present invention relates to a novel GIP analogue with the general amino acid sequence shown by formula (3) of

5 Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Tyr-Met
(3)

In another embodiment, the present invention provides novel GIP analogues of
10 formulas 4a-4l as result of an alanine scan. In particular, these are

Ala-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met
(4a)

15 Tyr-Ala-Ala-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met
(4b)

Tyr-Ala-Glu-Ala-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met
(4c)

Tyr-Ala-Glu-Gly-Ala-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met
(4d)

20 Tyr-Ala-Glu-Gly-Thr-Ala-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met
(4e)

Tyr-Ala-Glu-Gly-Thr-Phe-Ala-Ser-Asp-Tyr-Ser-Ile-Ala-Met
(4f)

25 Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ala-Asp-Tyr-Ser-Ile-Ala-Met
(4g)

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Ala-Tyr-Ser-Ile-Ala-Met
(4h)

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Ala-Ser-Ile-Ala-Met
(4i)

30 Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ala-Ile-Ala-Met
(4j)

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ala-Ala-Met

(4k)

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Ala

(4l)

5

Novel GIP analogues can be obtained by synthesis of linker peptides. Therefore, the present invention provides linker peptides according to formula (5):

10 Tyr-A-B-Gly-Thr-Phe-C-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-
Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln

(5)

wherein C is

15

a) not used,

b) a linker peptide consisting of 4 amino acid residues. Any combination of amino acid residues, including residues of D-amino acids and non-proteinogenic amino acids, is allowed and within the scope of the present invention,

20

c) Glu-Lys-Glu-Lys,

d) Ala-Ala-Ala-Ala,

e) a linker peptide consisting of 12 amino acid residues. Any combination of amino acid residues, including residues of D-amino acids and non-proteinogenic amino acids, is allowed and within the scope of the present invention,

25

f) Glu-Lys-Glu-Glu-Lys-Glu-Lys-Glu-Glu-Lys-Glu-Lys,

g) 6-Ahx_n (6-aminohexanoic acid) with n=1-3, or

f) an omega-amino fatty acid (saturated and/or unsaturated) with 6 to 34 carbon atoms, preferably 6 to 21 carbon atoms;

30

and wherein A and B are amino acid residues including D-amino acid residues, N-methylated amino acid residues and any other non-proteinogenic amino acid residues.

- 5 The N-terminus of the tyrosine residue in position 1 can be modified by alkylation, sulphonylation, glycation, homoserine formation, pyroglutamic acid formation, disulphide bond formation, deamidation of asparagine or glutamine residues, methylation, t-butylation, t-butyloxycarbonylation, 4-methylbenzylation, thioanysilation, thiocresylation, benzyloxymethylation, 4-nitrophenylation, benzyloxycarbonylation, 2-nitrobenzoylation, 2-nitrosulphonylation, 4-toluenesulphonylation, pentafluorophenylation, 10 diphenylmethylation, 2-chlorobenzyloxycarbonylation, 2,4,5-trichlorophenylation, 2-bromobenzyloxycarbonylation, 9-fluorenylmethyloxycarbonylation, triphenylmethylation, 2,2,5,7,8-pentamethylchroman-6-sulphonylation, hydroxylation, oxidation of methionine, 15 formylation, acetylation, anisylation, benzylation, benzoylation, trifluoroacetylation, carboxylation of aspartic acid or glutamic acid, phosphorylation, sulphation, cysteinylolation, glycolysation with pentoses, deoxyhexoses, hexosamines, hexoses or N-acetylhexosamines, farnesylation, 20 myristoylation, palmitoylation, stearoylation, geranylgeranylation, glutathionylation, 5'-adenosylation, ADP-ribosylation, modification with N-glycolylneuraminic acid, N-acetylneuraminic acid, pyridoxal phosphate, lipoic acid, 4'-phosphopantetheine, and N-hydroxysuccinimide. Further, the introduction of a reduced peptide bond or any other modification of the peptide 25 bond between position 2 and 3 is provided. The peptide of formula 5 can be modified by the introduction of at least one ϵ -amino fatty acid acylated lysine in any amino acid position.

Further, the present invention provides linker peptides according to formula (6):

Tyr-A-B-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-D-Gln-Gln-Asp-Phe-Val-
Asn-Trp-Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-
Gln

5

(6)

wherein D is

- g) not used,
- h) a linker peptide consisting of 4 amino acid residues. Any combination of amino acid residues, including residues of D-amino acids and non-proteinogenic amino acids, is possible and within the scope of the present invention, is allowed and within the scope of the present invention.
- i) Ala-Ala-Ala-Ala,
- j) Glu-Lys-Glu-Lys
- 15 k) 6-Ahx_n (6-aminohexanoic acid) with n=1-3, or
- l) an omega-amino fatty acid (saturated and/or unsaturated) with 6 to 34 carbon atoms, preferably 6 to 21 carbon atoms;

20

wherein A and B are amino acid residues including D-amino acid residues, N-methylated amino acid residues and any other non-proteinogenic amino acid residues.

25

The N-terminus of the tyrosine residue in position 1 can be modified by alkylation, acetylation and glycation. Further, the introduction of a reduced peptide bond or any other modification of the peptide bond between position 2 and 3 is provided. The peptide of formula 6 can be modified by the introduction of at least one ϵ -amino fatty acid acylated lysine in any amino acid position.

30

Other novel GIP analogues can be obtained by phosphorylation of Ser². Preferred compounds of the present invention are those of formulas 7a-7c:

Novel GIP analogues of formulas 7a-7c, comprising a phosphorylated seryl residue:

Tyr-[Ser(P)]-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met

(7a)

5 Tyr-[Ser(P)]-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-His-
Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys

(7b)

10 Tyr-[Ser(P)]-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-His-
Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-
Lys-His-Asn-Ile-Thr-Gln

(7c)

15

Further, novel GIP analogues are constrained GIP analogues by introduction of
side-chain lactam bridges between Asp/Glu- and Lys- residues of the peptide
sequence. One preferred compound of the present invention is [Cyclo(Lys¹⁸;
20 Asp²¹)] GIP (1-30) as of formula 8

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-
Asp-Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys

25

(8)

30 The present invention further includes within its scope both the amide and the
free carboxylic acid forms of the compounds of this invention. In view of the
close relationship between the free compounds and the compounds in the form
of their amides, whenever a compound is referred to in this context, the amide

as well as the free carboxylic acid form is intended, provided such is possible or appropriate under the circumstances.

The compounds of the present invention can be converted into acid addition salts, especially pharmaceutically acceptable acid addition salts. The pharmaceutically acceptable salt generally takes a form in which an amino acids basic side chain is protonated with an inorganic or organic acid. Representative organic or inorganic acids include hydrochloric, hydrobromic, perchloric, sulfuric, nitric, phosphoric, acetic, propionic, glycolic, lactic, succinic, maleic, fumaric, malic, tartaric, citric, benzoic, mandelic, methanesulfonic, hydroxyethanesulfonic, benzenesulfonic, oxalic, pamolic, 2-naphthalenesulfonic, p-toluenesulfonic, cyclohexanesulfamic, salicylic, saccharinic or trifluoroacetic acid. All pharmaceutically acceptable acid addition salt forms of the compounds of the present invention are intended to be embraced by the scope of this invention.

In view of the close relationship between the free compounds and the compounds in the form of their salts, whenever a compound is referred to in this context, a corresponding salt is also intended, provided such is possible or appropriate under the circumstances.

The present invention further includes within its scope prodrugs of the compounds of this invention. In general, such prodrugs will be functional derivatives of the compounds which are readily convertible *in vivo* into the desired therapeutically active compound. Thus, in these cases, the methods of treatment of the present invention, the term "administering" shall encompass the treatment of the various disorders described with prodrug versions of one or more of the claimed compounds, but which converts to the above specified compound *in vivo* after administration to the subject. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", ed. H. Bundgaard, Elsevier, 1985 and the patent applications DE 198 28 113 and DE 198 28 114, which are fully incorporated herein by reference.

Where the compounds according to this invention have at least one chiral center, they may accordingly exist as enantiomers. Where the compounds possess two or more chiral centers, they may additionally exist as diastereomers. It is to be understood that all such isomers and mixtures thereof are encompassed within the scope of the present invention. Furthermore, some of the crystalline forms of the compounds may exist as polymorphs and as such are intended to be included in the present invention. In addition, some of the compounds may form solvates with water (i.e. hydrates) or common organic solvents, and such solvates are also intended to be encompassed within the scope of this invention.

The compounds, including their salts, can also be obtained in the form of their hydrates, or include other solvents used for their crystallization.

Several compounds of the present invention, including their corresponding pharmaceutically acceptable salts, are characterized in that they have an improved resistance against degradation by the enzyme activity of dipeptidyl peptidase IV (DP IV) or DP IV-like enzymes. DP IV is present in a wide variety of mammalian organs and tissues e.g. the intestinal brush-border (Gutschmidt S. et al., "In situ" - measurements of protein contents in the brush border region along rat jejunal villi and their correlations with four enzyme activities. Histochemistry 1981, 72 (3), 467-79), exocrine epithelia, hepatocytes, renal tubuli, endothelia, myofibroblasts (Feller A.C. et al., A monoclonal antibody detecting dipeptidyl peptidase IV in human tissue. Virchows Arch. A. Pathol. Anat. Histopathol. 1986; 409 (2):263-73), nerve cells, lateral membranes of certain surface epithelia, e.g. Fallopian tube, uterus and vesicular gland, in the luminal cytoplasm of e.g., vesicular gland epithelium, and in mucous cells of Brunner's gland (Hartel S. et al., Dipeptidyl peptidase (DPP) IV in rat organs. Comparison of immunohistochemistry and activity histochemistry. Histochemistry 1988; 89 (2): 151-61), reproductive organs, e.g. cauda epididymis and ampulla, seminal vesicles and their secretions (Agrawal & Vanha-Perttula, Dipeptidyl peptidases in bovine reproductive organs and

secretions. *Int. J. Androl.* 1986, 9 (6): 435-52). In human serum, two molecular forms of dipeptidyl peptidase are present (Krepela E. et al., Demonstration of two molecular forms of dipeptidyl peptidase IV in normal human serum. *Physiol. Bohemoslov.* 1983, 32 (6): 486-96). The serum high molecular weight form of DP IV is expressed on the surface of activated T cells (Duke-Cohan J.S. et al., Serum high molecular weight dipeptidyl peptidase IV (CD26) is similar to a novel antigen DPPT-L released from activated T cells. *J. Immunol.* 1996, 156 (5): 1714-21). In one embodiment of the present invention, all molecular forms, homologues and epitopes of DP IV from all mammalian tissues and organs, also of those, which are undiscovered yet, are intended to be embraced by the scope of this invention.

Among the rare group of proline-specific proteases, DP IV was originally believed to be the only membrane-bound enzyme specific for proline as the penultimate residue at the amino-terminus of the polypeptide chain. However, other molecules have been identified recently that are structurally non-homologous with DP IV, but exhibit corresponding enzyme activity. Among the DP IV-like enzymes identified so far are fibroblast activation protein α , dipeptidyl peptidase IV β , dipeptidyl aminopeptidase-like protein, N-acetylated α -linked acidic dipeptidase, quiescent cell proline dipeptidase, dipeptidyl peptidase II, attractin and dipeptidyl peptidase IV related protein (DPP 8), and these are described in the review article by Sedo & Malik (Sedo & Malik, Dipeptidyl peptidase IV-like molecules: homologous proteins or homologous activities? *Biochimica et Biophysica Acta* 2001, 36506: 1-10). In another preferred embodiment of the present invention, all molecular forms, homologues and epitopes of proteins comprising DP IV-like enzyme activity, from all mammalian tissues and organs, also of those, which are undiscovered yet, are intended to be embraced by the scope of this invention.

The common property of the compounds of the present invention, including their corresponding pharmaceutically acceptable salts, is their improved resistance against degradation by the enzyme activity of DP IV or DP IV like

enzymes that can be measured by MALDI-TOF mass spectrometry. The results for selected GIP analogues according to the present invention are shown in table 1 to example 3. It was demonstrated by MALDI-TOF-MS that the substitution of amino acids in the cleavage position by D-Ala², NMeGlu³, Pro³ or
5 the introduction of a reduced peptide leads to resistance against DPIV degradation for up to 24 hours in GIP₁₋₃₀ analogs as well as in the corresponding GIP₁₋₁₄ analogs. Analogs with Val-, Gly-, Ser-substitution for Ala² or D-Glu-substitution for Glu³ showed reduced hydrolysis rates by DPIV. For the results see also table 1.

Table 1: N-terminal sequences, masses and DP-IV-resistance of synthetic GIP analogs

GIP-analog	N-terminal sequence	Mass (M) calculated	MALDI M + H ⁺	half life after incubation with DP IV
GIP _{1-42a}	Tyr-Ala-Glu-Gly....	4983.64	4983.9	Not determined
GIP _{1-30a}	Tyr-Ala-Glu-Gly....	3552.02	3553.3	<15 min ^a
GIP _{3-42a}	Gln-Gly...	4749.38	4751.4	Not determined
D-Ala ² -GIP _{1-30a}	Tyr-D-Ala-Glu-Gly...	3552.02	3553.8	stable
N-MeGlu ³ -GIP _{1-30a}	Tyr-Ala-MeGlu-Gly....	3565.07	3566.1	stable
D-Glu ³ -GIP ₁₋₃₀	Tyr-Ala-D-Glu-Gly....	3551.07	3553.0	40.3 ± 4.8
Pro ³ -GIP ₁₋₃₀	Tyr-Ala-Pro-Gly....	3519.07	3522.9	stable
Ser ² -GIP _{1-30a}	Tyr-Ser-Glu-Gly....	3567.07	3568.0	137.1 ± 12.3
Val ² -GIP _{1-30a}	Tyr-Val-Glu-Gly....	3579.12	3580.7	298.3 ± 92.2
Gly ² -GIP _{1-30a}	Tyr-Gly-Glu-Gly....	3537.04	3539.1	150.5 ± 27.3
YAψ(CH ₂ NH)-GIP _{3-30a}	Tyr-Alaψ(CH ₂ NH)- Glu-Gly...	3537.07	3539.0	stable
GIP _{1-6a}	Tyr-Ala-Glu-Gly....	685.74	686.9	> 7.5 min
D-Ala ² -GIP _{1-6a}	Tyr-D-Ala-Glu-Gly....	685.74	686.7	stable
Gly ² -GIP _{1-6a}	Tyr-Gly-Glu-Gly....	671.71	672.0	Not detectable ^b
Ser ² -GIP _{1-6a}	Tyr-Ser-Glu-Gly....	701.74	702.0	79.0 ± 12.2
Pro ² -GIP _{1-6a}	Tyr-Pro-Glu-Gly....	711.78	712.7	> 7.5 min
Val ² -GIP _{1-6a}	Tyr-Val-Glu-Gly....	713.79	715.2	Not detectable
Pro ³ -GIP _{1-6a}	Tyr-Ala-Pro-Gly....	653.78	655.0	stable
YAψ(CH ₂ NH)-GIP _{3-14a}	Tyr-Alaψ(CH ₂ NH)- Glu-Gly...	1553.75	1555.7	stable
Pro ³ -GIP ₁₋₁₄	Tyr-Ala-Pro-Gly....	1535.75	1534.0	stable
D-Ala ² -GIP ₁₋₁₄	Tyr-D-Ala-Glu-Gly...	1567.75	1570.6	stable
GIP ₁₋₁₃	Tyr-Ala-Glu-Gly....	1435.57	1435.6	11.5 ± 2.5
GIP ₁₋₁₅	Tyr-Ala-Glu-Gly....	1681.85	1682.6	35.0 ± 5.2
GIP _{15-30a}	Asp-Lys-Ile-Arg....	2001.34	2003.3	Not determined
GIP _{17-30a}	Ile-Arg-Gln-Gln	1758.07	1761.1	Not determined
GIP _{19-30a}	Gln-Gln-Asp-Phe	1488.72	1489.8	Not determined
GIP _{7-30a}	Ile-Ser-Asp-Tyr	2882.31	2886.9	130.1 ± 10.6

5 ^a After 15 min are 92% of GIP₁₋₃₀ hydrolyzed

^b After 1500 min only 25 % of G²GIP₁₋₃₀ are degraded

10 In another preferred embodiment, the compounds of the present invention, including their corresponding pharmaceutically acceptable salts, are characterized by their ability to bind to the GIP-receptor. The ability of the compounds of the present invention, including their corresponding pharmaceutically acceptable salts to bind to the GIP-receptor can be measured employing binding studies using ¹²⁵I-labeled spGIP₁₋₄₂ such as pursuant to the method described in example 4.

15 The displacement studies do not show non-specific binding of the compounds to the receptor. This is a term used to describe binding remaining in the

presence of excess ($\geq 1 \mu\text{M}$) GIP₁₋₄₂ (or GIP₁₋₃₀). This value has already been subtracted from data presented.

5 Examples of compounds of the present invention that bind and displace ¹²⁵I-GIP₁₋₄₂ from the GIP-receptor are shown in Figures 8, 10 and 11 and in Tables 2 and 3.

10 Surprisingly, the compounds of the present invention are functionally active. The biological activity of the compounds of the present invention, including their corresponding pharmaceutically acceptable salts, can be measured by determining the production of cyclic AMP following receptor binding. The cAMP production assay is described in example 4. Substitution of D-Glu for Glu³ and D-Ala for Ala² resulted in peptides with only small reductions in their ability to stimulate adenylyl cyclase whereas the Val²- and Gly²-analogs showed a
15 significant reduction in efficacy. Interestingly, the introduction of the reduced peptide bond resulted in a dramatic deterioration of cAMP production. This confirms the importance of the integrity of the N-terminus of GIP. Further results are shown in Tables 2 and 3 and in Figures 1-7.

Table 2: Cyclic AMP production and competitive binding displacement studies on GIP analogs of variable length

Synthetic Peptide:	cAMP Production (Fold Basal ^a)		Receptor Binding	
	10 μ M	20 μ M	% Displacement at 10 μ M	IC ₅₀ (nM)
GIP(1-42) _{OH}	119 \pm 11	-	100	3.2 \pm 0.3
1-6 _{NH2}	1.27 \pm 0.18	1.08 \pm 0.03	-3.6 \pm 7.8	-
1-7 _{NH2}	0.92 \pm 0.05	1.06 \pm 0.06	-6.1 \pm 3.4	-
1-13 _{OH}	1.03 \pm 0.08	1.15 \pm 0.07	-0.2 \pm 3.4	-
1-13 _{NH2}	6.51 \pm 1.33	15.7 \pm 3.0	5.0 \pm 1.1*	-
1-14 _{OH}	88.9 \pm 9.5	85.2 \pm 7.6	51.3 \pm 1.2	-
1-14 _{NH2}	75.4 \pm 10.7	88.3 \pm 5.9	27.9 \pm 2.8	-
1-15 _{OH}	0.97 \pm 0.06	0.91 \pm 0.05	-3.1 \pm 4.3	-
1-15 _{NH2}	2.26 \pm 0.32	4.37 \pm 0.51*	4.2 \pm 1.7	-
1-30 _{NH2}	108 \pm 12	- ^c	99.8 \pm 1.2	2.0 \pm 0.7
7-30 _{NH2}	0.89 \pm 0.06	0.85 \pm 0.03	99.3 \pm 1.0	23.7 \pm 3.7
15-42 _{OH}	1.02 \pm 0.10	1.01 \pm 0.03	83.3 \pm 0.7	1270 \pm 150
15-30 _{NH2}	1.24 \pm 0.28	1.01 \pm 0.11	82.7 \pm 1.0	1400 \pm 310
16-30 _{NH2}	1.04 \pm 0.06	0.80 \pm 0.02	82.1 \pm 1.9	2530 \pm 450
17-30 _{NH2}	1.13 \pm 0.09	1.12 \pm 0.05	81.9 \pm 2.1	1540 \pm 550
19-30 _{NH2}	20.1 \pm 1.3	45.0 \pm 1.6	52.3 \pm 0.6	-

*: $p < 0.05$

^a: Basal cyclic AMP = 2.737 ± 0.079 fmol/1000 cells;

^b: cyclic AMP stimulated by 20 μ M peptide, if plateau levels were not achieved

^c: By definition, 10 μ M GIP₁₋₄₂ displaces all specific ¹²⁵I-GIP binding.

^d: Estimated using maximal GIP₁₋₄₂-stimulated cAMP.

Table 3: Summary statistics for cyclic AMP production and competitive binding displacement studies on synthetic GIP fragments using CHO-K1 cells transfected with the rat GIP-receptor. Data represent mean \pm S.E.M. of 3 independent experiments.

Synthetic Peptide:	Molecular Weight (Daltons)		cAMP Production		Receptor Binding	
	Expected	Measured	Max. cAMP ^a (Fold Basal)	EC ₅₀	% Displacement at 20 μ M	IC ₅₀
GIP (1-42OH)	4984.3	4984.7	122 \pm 10	231 \pm 34 pM	100 ^c	4.18 \pm 0.47 nM
GIP (1-6)(19-42)NH ₂	2157.8	2158.8	1.81 \pm 0.42 ^a	-	88.2 \pm 0.7	2.74 \pm 0.37 \square M
GIP (1-6)(AAAA)(19-42)NH ₂	2441.8	2440.5	7.21 \pm 0.99 ^a	-	88.7 \pm 3.0	2.41 \pm 0.48 \square M
GIP (1-6)(EKEQ)(19-42)NH ₂	2672.1	2674.1	8.17 \pm 0.87 ^a	-	86.8 \pm 1.8	2.09 \pm 0.23 \square M
GIP (1-6)(EKEEKEEKEE)(19-42)NH ₂	3574.0	3575.9	84.9 \pm 8.1 ^a	8.39 \pm 0.18 μ M ^{cd}	75.1 \pm 2.7	4.27 \pm 0.14 \square M
GIP (1-6)(AAd)(19-42)NH ₂	2270.7	2274.0	95.9 \pm 8.8 ^a	14.6 \pm 4.7 μ M ^{cd}	62.0 \pm 4.3	8.73 \pm 2.24 \square M
GIP (1-6)(AAd)(19-42)NH ₂	2383.8	2386.0	2.55 \pm 0.84 ^a	-	75.9 \pm 3.3	4.98 \pm 0.40 \square M
GIP (1-6)(AAd)(19-42)NH ₂	2497.0	2498.8	13.5 \pm 1.5 ^a	-	67.1 \pm 1.0	4.03 \pm 0.64 \square M
GIP (1-6)(19-42)NH ₂	3038.6	3040.8	127 \pm 22	78.7 \pm 2.3 nM ^a	95.4 \pm 0.7	1.97 \pm 0.08 \square M
GIP (1-14)(AAAA)(19-42)NH ₂	3322.9	3328.3	82.1 \pm 2.8 ^a	58.7 \pm 2.7 pM ^a	100.0 \pm 0.9	65.3 \pm 7.5 nM
GIP (1-14)(EKEQ)(19-42)NH ₂	3553.0	3551.8	80.8 \pm 5.8 ^a	77.0 \pm 8.1 pM ^a	98.7 \pm 1.1	26.0 \pm 1.8 nM
GIP (1-14)(AAd)(19-42)NH ₂	3151.6	3155.8	102.1 \pm 5.0	1.41 \pm 0.32 μ M ^{cd}	88.1 \pm 1.9	2.71 \pm 0.23 \square M
GIP (1-14)(AAd)(19-42)NH ₂	3264.9	3264.8	95.9 \pm 3.2 ^a	2.51 \pm 0.25 μ M ^{cd}	85.8 \pm 1.6	2.77 \pm 0.14 \square M
GIP (1-14)(AAd)(19-42)NH ₂	3377.9	3389.4	49.5 \pm 1.6 ^a	~ 20 μ M ^{cd}	82.7 \pm 3.2	3.21 \pm 0.44 \square M

*: p < 0.05

a. Basal cyclic AMP = 2.737 \pm 0.079 fmol/1000 cells;

b. cyclic AMP stimulated by 20 μ M peptide, if plateau levels were not achieved

c. By definition, 10 μ M GIP₁₋₄₂ displaces all specific ¹²⁵I-GIP binding.

d. Estimated using maximal GIP₁₋₄₂-stimulated cAMP.

Based on their functional activity *in vitro*, compounds of the present invention were tested for their ability to improve glucose tolerance and decrease glucose AUC in mammals *in vivo* and therefore are useful for the treatment of non-insulin dependent diabetes mellitus (NIDDM). The ability of the compounds, including their corresponding pharmaceutically acceptable salts, to improve glucose tolerance in a mammal and to decrease glucose AUC can be measured employing the Wistar rat model. The method is described in Example 5. Results are shown in Figures 12, 13 and 14.

Based on their receptor binding capabilities and their stimulatory effect on cAMP release, it was found that the compounds of the present invention are able to potentiate glucose dependent proliferation of pancreatic β -cells. Surprisingly, and as an especially preferred embodiment, the compounds of the present invention show, independently from the presence of glucose, a

concentration-dependent effect on the β -cell survival. The ability of the compounds of the present invention, including their corresponding pharmaceutically acceptable salts, to potentiate glucose dependent β -cell proliferation as well as glucose independent β -cell survival can be measured
5 employing an assay with INS-1 cells as described in Example 6. Results are shown in Figures 15 and 16.

One of the most surprising findings is that the compounds of the present invention have an anti-apoptotic effect on pancreatic β -cells. The anti-apoptotic
10 effect of the compounds of the present invention, including their corresponding pharmaceutically acceptable salts, can be measured employing a caspase-3 activation assay as described in Example 7. The results are shown in figure 18A. Caspase-3 activation is a marker for the induction of cellular apoptosis. Based on their receptor binding capabilities and their stimulatory effect on
15 cAMP release, it was found that the compounds of the present invention are able to selectively block activation of caspase-3 in response to glucose withdrawal.

In another *in vitro* assay, streptozotocin (STZ)-induced β -cell death of INS-1
20 cells, it has been demonstrated that the compounds of the present invention and including their corresponding pharmaceutically acceptable salts, are able to protect against the pro-apoptotic (caspase-3 activating) effects of STZ completely. The method is described in Example 7. The results are shown in Figure 18B.

25 In a further embodiment, the present invention provides pharmaceutical compositions e.g. useful in GIP-receptor binding comprising a pharmaceutically acceptable carrier or diluent and a therapeutically effective amount of a compound of formulas 1-8, or a pharmaceutically acceptable salt thereof.

30 In still another embodiment, the present invention provides a method for binding or blocking GIP-receptor comprising administering to a mammal in need of such

treatment a therapeutically effective amount of a compound of formulas 1-8 above, or a pharmaceutically acceptable salt thereof.

In a further embodiment, the present invention provides a method for treating
5 conditions mediated by GIP-receptor binding comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound of formulas 1-8 above, or a pharmaceutically acceptable salt thereof.

The present invention also relates to the use of a compound according to the
10 present invention or a pharmaceutically acceptable salt thereof e.g. for the manufacture of a medicament for the prevention or treatment of diseases or conditions associated with GIP-receptor signaling.

In a preferred embodiment, the present invention relates to the use of a
15 compound according to the present invention or a pharmaceutically acceptable salt thereof e.g. for the manufacture of a medicament for the prevention or treatment of diabetes mellitus and obesity.

Examples of the invention

20

Example 1: Solid-phase Synthesis of Peptides

The GIP analogs were synthesized with an automated synthesizer SYMPHONY (RAININ) using a modified Fmoc-protocol. Cycles were modified by using
25 double couplings from the 15th amino acid from the C-terminus of the peptide with five-fold excess of Fmoc-amino acids and coupling reagent. The peptide couplings were performed by TBTU/NMM-activation using a 0.23 mmol substituted NovaSyn TGR-resin or the corresponding preloaded Wang-resin at 25 μ mol scale. The cleavage from the resin was carried out by a cleavage-cocktail consisting of 94.5 % TFA, 2.5 % water, 2.5 % EDT and 1 % TIS.
30

Analytical and preparative HPLC were performed by using different gradients on the LiChrograph HPLC system of Merck-Hitachi. The gradients were made up from two solvents: (A) 0.1 % TFA in H₂O and (B) 0.1 % TFA in acetonitrile.

Analytical HPLC were performed under the following conditions: solvents were run (1 ml/min) through a 125-4 Nucleosil RP18-column, over a gradient from 5 % - 50 % B over 15 min and then up to 95 % B until 20 min, with UV detection ($\lambda = 220$ nm). Purification of the peptides was carried out by preparative HPLC on either a 250-20 Nucleosil 100 RP8-column or a 250-10 LiChrospher 300 RP18-column (flow rate 6 ml/min, 220 nm) under various conditions depending on peptide chain length.

For the identification of the peptide analogues, laser desorption mass spectrometry was employed using the HP G2025 MALDI-TOF system of Hewlett-Packard.

Example 2: Synthesis of GIP analogues with a reduced peptide bond

Tyr-Ala ψ (CH₂NH)-GIP_{3-30a} and Tyr-Ala ψ (CH₂NH)-GIP_{3-14a} were synthesized by coupling 2 equivalents of Fmoc-Tyr(tBu) ψ (CH₂NH)-Glu(tBu)-Gly-OH by TBTU/DIPEA activation and double coupling over 4 hours. The corresponding GIP₅₋₃₀ and GIP₅₋₁₄ fragments were synthesized as described above.

The synthesis of the fully protected tetrapeptide Tyr-Ala ψ (CH₂NH)-Glu(tBu)-Gly-OH was carried out on the acid sensitive Sasrn resin in a 0.7 mmol scale by Fmoc-strategy as described in Example 1 using a half-automated peptide synthesizer Labortec (BACHEM). The protected tetrapeptide was cleaved from the resin by 1 % TFA. The reduced peptide bond was incorporated via reductive alkylation of the N-terminal deprotected peptide on the sasrn resin with Fmoc-alaninal.

Example 3: Determination of DPIV Resistance by MALDI-TOF Mass Spectrometry

The hydrolysis of peptide analogues by purified kidney DPIV was studied as described previously [12]. In brief, peptides were incubated in 0.04 M Tris buffer pH 7.6 and DPIV for up to 24 h. Samples were removed from the incubation

mixture and prepared for MALDI-TOF mass spectrometry, as described in Pauly, R. P., Rosche, F., Wermann, M., McIntosh, C. H. S., Pederson, R. A., and Demuth, H. U. Investigation of glucose-dependent Insulinotropic polypeptide-(1- 42) and glucagon-like peptide-1-(7-36) degradation in vitro by dipeptidyl peptidase IV using matrix-assisted laser desorption/ionization time of flight mass spectrometry - A novel kinetic approach. J Biol Chem 271(38), 23222-23229. 1996.

10 *Example 4: In vitro studies*

Chinese hamster ovary (CHO-K1) cells stably expressing the rat pancreatic islet (wild type) GIP-receptor (wtGIP-R1 cells) were prepared as described previously [19,21]. Cells were cultured in DMEM/F12, supplemented with 10 % newborn calf serum, 50 units/ml penicillin G, and 50 µg/ml streptomycin (Culture media and antibiotics from Gibco BRL, Life Technologies). Cells were grown in 75 cm² flasks until 80-90 % confluent, when they were split and seeded onto 24 well plates at a density of 50,000 cells/well. Experiments were carried out 48 h later.

20

Binding studies

Binding studies using ¹²⁵I-labeled spGIP₁₋₄₂, purified by high performance liquid chromatography (HPLC), were performed essentially as described previously [21]. wtGIP-R1 Cells (1-5 x 10⁵/well) were washed twice at 4 °C in binding buffer (BB), consisting of DMEM/F12 (GIBCO), 15 mM HEPES, 0.1 % bovine serum albumin (BSA), 1 % Trasylol (aprotinin; Bayer), pH 7.4. They were incubated for 12-16 h at 4 °C with ¹²⁵I-spGIP (50,000 cpm) in the presence or absence of unlabeled GIP₁₋₄₂ or analogue. Following incubation, cells were washed twice with ice cold buffer, solubilized with 0.1 M NaOH (1 ml), and transferred to culture tubes for counting of cell-associated radioactivity. Nonspecific binding was defined as that measured in the presence of 1 µM GIP₁₋₄₂ or GIP₁₋₃₀, and specific binding expressed as % of binding in the absence of competitor (%B/Bo).

cAMP production

Wild type GIP-R1 cells were cultured for 48 h, washed in BB at 37 °C, and preincubated for 1 h prior to a 30 min stimulation period with test agents in the presence of 0.5 mM IBMX (Research Biochemicals Intl., Natick, MA) [19,21]. With inhibition experiments, cells were incubated with GIP analogues for 15 min prior to a 30 min stimulation with 1 nM shGIP₁₋₄₂. Cells were extracted with 70 % ethanol and cAMP levels measured by radioimmunoassay (Biomedical Technologies, Stoughton, MA) [19,21]. Data are expressed as fmol/1000 cells or % maximal GIP₁₋₄₂-stimulated cAMP production (inhibition experiments).

Example 5: Improvement of glucose tolerance after subcutaneous administration of synthetic GIP analogues to Wistar rats

15

Male Wistar rats (250-350 g) were starved overnight (16-18 hours) with free access to drinking water. Whole blood samples were taken from the tail vein of conscious unrestrained rats, for determination of blood glucose (using a hand-held glucometer); plasma was separated by centrifugation (20 min, 12,000 rpm, 4°C) for measurement of plasma insulin concentrations. A basal sample was obtained immediately prior to an oral glucose tolerance test (1 gram glucose/Kg body weight) and intra-scapular subcutaneous injection of peptide analogue (8 nmol/Kg body weight) or saline control (500 microlitre injection volume). Blood samples were taken at t = 2, 10, 20, 30, and 60 for insulin determination, and blood glucose was measured at 10 minute intervals. Integrated glucose response was calculated using the trapezoidal algorithm with baseline subtraction.

30 *Example 6: GIP stimulates cell proliferation and promotes survival of β -(INS-1) Cells*

Cell culture and reagents

INS-1 cells (clone 832/13) were cultured in 11 mM glucose RPMI (Sigma Laboratories, Natick, MA, USA) supplemented with 2 mM glutamine, 50 μ M β -mercaptoethanol, 10 mM HEPES, 1 mM sodium pyruvate, and 10 % fetal bovine serum (Cansera, Rexdale, Ont., Canada). Prior to experiments, cells were harvested into either 6-well (2×10^6 cells/well; Becton Dickinson, Lincoln Park, NJ, USA), 24-well (5×10^5 cells/well), or 96-well (5×10^4 cells/well) plates. Cell passages 45-60 were used.

GIP-receptor characterization studies; competitive binding, cAMP, and insulin release

Synthetic porcine GIP (5 μ g) was iodinated by the chloramine-T method, and the 125 I-GIP was further purified by reverse phase high performance liquid chromatography to a specific activity of 250-300 μ Ci/ μ g. Competitive binding analyses were performed as described in Example 4. For cAMP studies, cells were washed twice and then stimulated for 30 minutes with GIP in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (0.5 mM IBMX; RBI/Sigma, Natick, MA, USA). Following stimulation, reactions were stopped, and cells lysed, in 70% ice-cold ethanol, cellular debris removed by centrifugation, and cAMP subsequently quantified by radioimmunoassay (RIA) (Biomedical Technologies Inc., Stoughton, MA, USA). All insulin release experiments were performed over 60 minutes, in the absence of IBMX, and insulin secreted into the media was quantified by RIA.

Since GIP-receptors in the INS-1 clone 832/13 cell line had not been previously characterized, binding, adenylyl cyclase stimulation and insulin secretory responses to GIP were initially studied. Cells expressed receptors at a density of 1571 ± 289 binding sites/cell ($n=3$) with an IC_{50} for binding of 21.1 ± 2.49 nM ($n=3$) and a $K_D = 106.2 \pm 4.3$ fmol ($n=3$); cAMP production was stimulated by GIP with an EC_{50} of 4.70 ± 1.81 nM ($n=4$); 5.5 mM glucose stimulated insulin secretion was potentiated by 10 nM GIP (1.63 ± 0.183 % total insulin secreted for 5.5 mM glucose vs. 2.44 ± 0.29 % total insulin secreted ($p < 0.05$, $n=3$)).

Cell quantification

Cells were seeded into 96-well plates (5×10^4 cells/well) prior to experimentation. After establishing metabolic quiescence in the absence of serum for 24 h, cells were cultured in low glucose media (RPMI with 0.1 % BSA) with agonists (glucose, glucose + GIP/GLP-1/GH) for an additional 24 h. Thereafter, cells were washed with KRBH (115 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4 , 10 mM NaHCO_3 , 1.28 mM CaCl_2 , 1.2 mM MgSO_4 containing 10 mM HEPES and 0.1 % bovine serum albumin, pH 7.4) and frozen at -70°C until assayed. Cells were quantified using the CYQUANT™ assay system (Molecular Probes, Eugene, OR, USA) according to the manufacturers' protocol. Final cell numbers were always greater than the initial number plated in assessing cellular proliferation.

Cell survival was assessed in the presence of prolonged glucose deprivation. 24 h after glucose deprivation (RPMI with 0.1 % BSA), GIP or forskolin were added for an additional 24 h, and cell number was quantified. Final cell numbers were always less than the initial number plated in assessing cell survival.

GIP potentiates glucose dependent β -cell proliferation

The INS-1 cell line has been extensively investigated previously as a cellular model for β -cell proliferation (Hugl SR, White MF, Rhodes CJ 1998: Insulin-like growth factor 1 (IGF-1)-stimulated pancreatic beta-cell growth is glucose-dependent. J. Biol. Chem. 273:17771-17779; Dickson LM, Linghor MK, McCuaig J, Hugl SR, Snow L, Kahn BB, Myers Jr. MG, Rhodes CJ (2001), Differential activation of protein kinase B and p70S6K by glucose and insulin-like growth factor 1 in pancreatic beta cells (INS-1). J. Biol. Chem. 276:21110-21120). GIP was found to potentiate 11 mM glucose mediated β -cell proliferation (Figure 15A) to levels comparable to those obtained with GH (158 ± 16 % of growth in the presence of 5.5 mM glucose for 100 nM GIP; 158 ± 9 % for 10 nM GH ($n=3-5$)). In a separate experiment (Fig. 16B), 100 nM GIP stimulated cell growth to 131 ± 7 % of that measured in the presence of 5.5 mM

glucose, similar to the proliferative responses to 100 nM GLP-1 ($129 \pm 4 \%$; $n=4$).

GIP reverses the detrimental effect of 0 mM glucose

5 While determining the glucose-dependence of these growth promotive effects, it was observed that GIP was capable of reversing the detrimental effects of 0 mM glucose media on cellular survival. Incubation of cells in the presence of 0 mM glucose media for 48 h resulted in approximately 50 % cell death (Figure 16A). Surprisingly, $91 \pm 10 \%$ of the cells plated remained viable when the
10 media was supplemented with 100 nM GIP after 24 h. These cell survival effects of GIP were found to be concentration-dependent with an EC_{50} value of 1.24 ± 0.48 nM GIP ($n=4$; Figure 16B).

GIP has a protective effect against wortmannin-induced cell death

15 In order to establish which intracellular signaling pathways were involved in the GIP-induced cell survival, studies were performed with pharmacological inhibitors used at concentrations shown to exhibit selectivity for candidate protein kinases (Figure 17). Stimulation of adenylyl cyclase with forskolin mimicked the effects of GIP on cell survival, but the lack of effect of H89 (Figs.
20 18A and B) indicates a PKA-independent mode of action. Neither of the Mek1/2 inhibitors PD98059 (50 and 100 μ M) nor U0126 (10 μ M) blocked the effects of GIP on cell survival ($n=3$). The ability of GIP to promote cell survival was further supported by studies on the effect of the PI3Kinase-PKB pathway inhibitor, wortmannin (Figure 17C). Since wortmannin alone promoted cell loss
25 it was not possible to determine whether GIP activates the PI3Kinase-PKB pathway. However, cells were partially protected against wortmannin-induced cell loss by GIP treatment ($n=3$, $p<0.05$). The only compound tested that influenced GIP-mediated cell survival was the inhibitor SB202190 (Figure 17D), indicating that GIP can act via p38 MAPK.

30

Example 7: GIP has an anti-apoptotic effect

Caspase-3 activity

INS-1 cells (clone 832/13) seeded into 6-well plates were serum starved for 12-24 h and subjected to glucose deprivation (RPMI with 0.1 % BSA) or treatment with 2 mM streptozotocin (STZ). GIP and GLP-1 were added 10 min prior to
5 STZ and for 30 min during STZ. Following treatment, caspase-3 activity was determined after 2, 6, or 24 h according to the manufacturers' protocol (Molecular Probes, Eugene, OR, USA). Caspase-3 activity/well was corrected for total protein content using the BCA protein assay (Pierce, Roxford, IL, USA).

10 Caspase-3 activation is a marker for the induction of cellular apoptosis. To establish whether the cell survival effects of GIP were due to anti-apoptotic actions of the polypeptide, activation of caspase-3 induced by glucose deprivation was studied. Figure 18A illustrates that 0 mM glucose promoted apoptosis by 6 h (not by 2 h; data not shown), and that this effect was
15 completely reversed by addition of GIP or forskolin. The conclusion that GIP selectively blocked activation of caspase-3 in response to glucose withdrawal was confirmed by the demonstration that the specific aldehyde inhibitor of caspase-3, Ac-DEVD-CHO, completely blocked low glucose activation (Fig. 19A).

20

STZ-induced cell death

The ability of GIP to protect against streptozotocin (STZ)-induced β -cell death was studied. When added 10 minutes prior to, and during, a 30 minute STZ exposure, GIP was able to protect against the pro-apoptotic (caspase-3
25 activating) effects of STZ completely (Figure 18B).

Claims

5

1. A compound having the amino acid sequence:

Tyr-A-B-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met

10

wherein A and B are amino acid residues including D-amino acid residues, N-methylated amino acid residues and any other non-proteinogenic amino acid residues or a pharmaceutically acceptable salt thereof, excluding the sequence of native GIP (1-14).

15

2. A compound according to claim 1, wherein the N-terminus of the tyrosine residue in position 1 can be modified by alkylation, sulphonylation, glycation, homoserine formation, pyroglutamic acid formation, disulphide bond formation, deamidation of asparagine or glutamine residues, methylation, t-butylation, t-butylloxycarbonylation, 4-methylbenzylation, thioanysilation, thiocresylation, benzyloxymethylation, 4-nitrophenylation, benzyloxycarbonylation, 2-nitrobenzylation, 2-nitrosulphenylation, 4-toluenesulphonylation, pentafluorophenylation, diphenylmethylation, 2-chlorobenzyloxycarbonylation, 2,4,5-trichlorophenylation, 2-bromobenzyloxycarbonylation, 9-fluorenylmethyloxycarbonylation, triphenylmethylation, 2,2,5,7,8-pentamethylchroman-6-sulphonylation, hydroxylation, oxidation of methionine, formylation, acetylation, anisylation, benzylation, benzoylation, trifluoroacetylation, carboxylation of aspartic acid or glutamic acid, phosphorylation, sulphation, cysteinilation, glycolysation with pentoses, deoxyhexoses, hexosamines, hexoses or N-acetylhexosamines, farnesylation, myristoylation, biotinylation, palmitoylation, stearylgeranylation, glutathionylation, 5'-adenosylation, ADP-ribosylation, modification with N-

20

25

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glycolylneuraminic acid, N-acetylneuraminic acid, pyridoxal phosphate, lipoic acid, 4'-phosphopantetheine, and N-hydroxysuccinimide.

3. A compound according to claims 1 or 2, wherein the peptide is modified by the introduction of at least one ϵ -amino fatty acid acylated lysine in any amino acid position.

4. A compound according to claims 1, 2 or 3 having the amino acid sequence:

10 Tyr-(D-Ala)-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met.

5. A compound according to claims 1, 2 or 3 having the amino acid sequence:

15 Tyr-Ala-Pro-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met.

6. A compound having the following amino acid sequence and comprising a reduced peptide bond:

20

Tyr-Ala- Ψ (CH₂NH₂)-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met; or

Tyr-Ala- ψ (CH₂NH)-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys; or

25

a pharmaceutically acceptable salt thereof.

7. A compound having the amino acid sequence:

30

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Tyr-Met

or a pharmaceutically acceptable salt thereof.

8. A compound having the amino acid sequence:

5

Ala-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met;

Tyr-Ala-Ala-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met;

10 Tyr-Ala-Glu-Ala-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met;

Tyr-Ala-Glu-Gly-Ala-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met;

Tyr-Ala-Glu-Gly-Thr-Ala-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met;

15

Tyr-Ala-Glu-Gly-Thr-Phe-Ala-Ser-Asp-Tyr-Ser-Ile-Ala-Met;

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ala-Asp-Tyr-Ser-Ile-Ala-Met;

20 Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Ala-Tyr-Ser-Ile-Ala-Met;

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Ala-Ser-Ile-Ala-Met;

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ala-Ile-Ala-Met;

25

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ala-Ala-Met; or

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Ala;

30 or a pharmaceutically acceptable salt thereof.

9. A compound having the following amino acid sequence and comprising one or more linker peptides:

Tyr-A-B-Gly-Thr-Phe-C-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-
5 Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln;

wherein C is

- a) not used,
- 10 b) a linker peptide comprising 4 amino acid residues selected from the group consisting of amino acid residues, D-amino acids and non-proteinogenic amino acids,
- c) Glu-Lys-Glu-Lys,
- d) Ala-Ala-Ala-Ala,
- 15 e) a linker peptide comprising 12 amino acid residues selected from the group consisting of amino acid residues, D-amino acids and non-proteinogenic amino acids,
- f) Glu-Lys-Glu-Glu-Lys-Glu-Lys-Glu-Glu-Lys-Glu-Lys,
- g) 6-Ahx_n (6-aminohexanoic acid) with n=1-3, or
- 20 h) an omega-amino fatty acid (saturated and/or unsaturated) with 6 to 34 carbon atoms; and

wherein A and B are amino acid residues, D-amino acid residues, N-methylated amino acid residues or any other non-proteinogenic amino acid residues; or
25 pharmaceutically acceptable salts thereof.

10. A compound according to claim 9, wherein the N-terminus of the tyrosine residue in position 1 can be modified by alkylation, sulphonylation, glycation, homoserine formation, pyroglutamic acid formation, disulphide bond formation,
30 deamidation of asparagine or glutamine residues, methylation, t-butylation, t-butyloxycarbonylation, 4-methylbenzylation, thioanysilation, thiocresylation, benzyloxymethylation, 4-nitrophenylation, benzyloxycarbonylation, 2-

nitrobenzoylation, 2-nitrosulphenylation, 4-toluenesulphonylation, pentafluorophenylation, diphenylmethylation, 2-chlorobenzyloxycarbonylation, 2,4,5-trichlorophenylation, 2-bromobenzyloxycarbonylation, 9-fluorenylmethyloxycarbonylation, triphenylmethylation, 2,2,5,7,8-pentamethylchroman-6-sulphonylation, hydroxylation, oxidation of methionine, formylation, acetylation, anisylation, benzylation, benzoylation, trifluoroacetylation, carboxylation of aspartic acid or glutamic acid, phosphorylation, sulphation, cysteinylolation, glycolysation with pentoses, deoxyhexoses, hexosamines, hexoses or N-acetylhexosamines, famesylation, myristolysation, blotinylation, palmitoylation, stearylation, geranylgeranylation, glutathionylation, 5'-adenosylation, ADP-ribosylation, modification with N-glycolylneuraminic acid, N-acetylneuraminic acid, pyridoxal phosphate, lipoic acid, 4'-phosphopantetheine, and N-hydroxysuccinimide.

11. A compound according to claims 9 or 10, wherein the peptide is modified by the introduction of at least one ϵ -amino fatty acid acylated lysine in any amino acid position.

12. A compound according to claims 9, 10 or 11, wherein the peptide is modified by introduction of a reduced peptide bond or any other modification of the peptide bond between A and B.

13. A compound having the following amino acid sequence and comprising one or more linker peptides:

Tyr-A-B-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-D-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln

wherein D is

a) not used,

- b) a linker peptide comprising 4 amino acid residues selected from the group consisting of amino acid residues, D-amino acids and non-proteinogenic amino acids,
- c) Ala-Ala-Ala-Ala,
- 5 d) Glu-Lys-Glu-Lys,
- e) 6-Ahx_n (6-aminohexanoic acid) with n=1-3, or
- f) an omega-amino fatty acid (saturated and/or unsaturated) with 6 to 34 carbon atoms,

10 wherein A and B are amino acid residues, D-amino acid residues, N-methylated amino acid residues or any other non-proteinogenic amino acid residues; and pharmaceutically acceptable salts thereof.

14. A compound according to claim 13, wherein the N-terminus of the tyrosine residue in position 1 can be modified by alkylation, acetylation or glycation.

15. A compound according to claims 13 or 14, wherein the peptide is modified by the introduction of at least one ϵ -amino fatty acid acylated lysine in any amino acid position.

20 16. A compound according to claims 13, 14 or 15, wherein the peptide is modified by introduction of a reduced peptide bond or other modification of the peptide bond between A and B.

25 17. A compound having the following amino acid sequence and comprising a phosphorylated seryl residue:

Tyr-[Ser(P)]-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met,

30 Tyr-[Ser(P)]-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys, or

Tyr-[Ser(P)]-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln; or

5 pharmaceutically acceptable salts thereof.

18. A compound having the amino acid sequence:

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-
10 Asp-Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys; or
a pharmaceutically acceptable salt thereof.

19. A compound according to any one of the preceding claims in free carboxylic acid form or a pharmaceutically acceptable salt thereof.

15

20. A compound according to any one of the preceding claims in amide form or a pharmaceutically acceptable salt thereof.

20

21. A compound according to any one of the preceding claims characterized in that the compound is resistant to the degradation by dipeptidyl peptidase IV or dipeptidyl peptidase IV-like enzyme activity.

25

22. A compound according to any one of the preceding claims characterized in that the compound is a GIP-receptor agonist.

23. A compound according to any one of claims 1 to 21 characterized in that the compound is a GIP-receptor antagonist.

30

24. A compound according to any one of the preceding claims characterized in that the compound potentiates cyclic AMP production.

25. A compound according to any one of the preceding claims characterized in that the compound blocks the activation of caspase-3.

26. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and a compound according to any one of the preceding
5 claims, or a pharmaceutically acceptable acid addition salt thereof.

27. Use of a pharmaceutical composition or a compound according to any one of the preceding claims or a pharmaceutically acceptable acid addition salt
10 thereof for the manufacture of a medicament for the prevention or treatment of diseases or conditions related to impaired binding of GIP- receptor analogues.

28. Use according to claim 27 for the manufacture of a medicament for the prevention or treatment of β -cell apoptosis.

15

29. Use according to claim 27 for the manufacture of a medicament for the potentiation of glucose dependent proliferation of pancreatic β -cells.

30. Use according to claim 27 for the manufacture of a medicament for the
20 treatment of non-insulin-dependent diabetes mellitus and obesity.

31. A method for treating conditions mediated by GIP-receptor binding comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound or pharmaceutical composition
25 according to any one of claims 1-26.

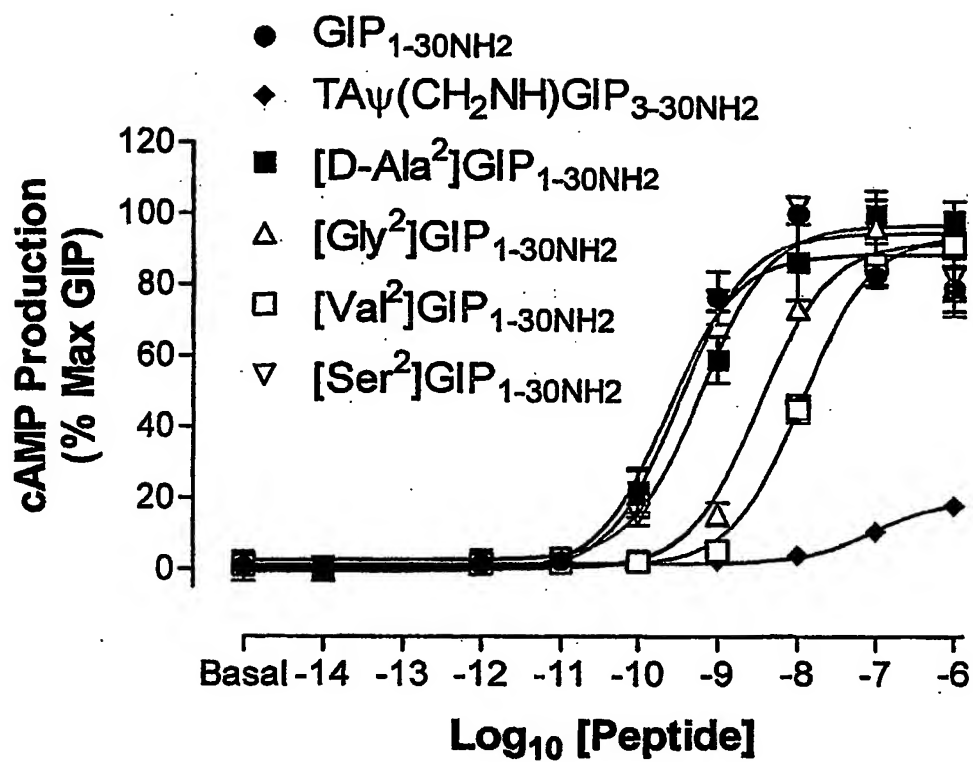
32. A method for lowering elevated blood glucose levels in mammals resulting from food intake comprising administering a therapeutically effective amount of

at least one compound or pharmaceutical composition according to any one of the claims 1-26.

33. A compound or pharmaceutical composition according to any one of claims
5 1-26, wherein the compound is modified by introduction of a side-chain lactam bridges between two amino acid residues of the peptide sequence, and pharmaceutically acceptable salts thereof.

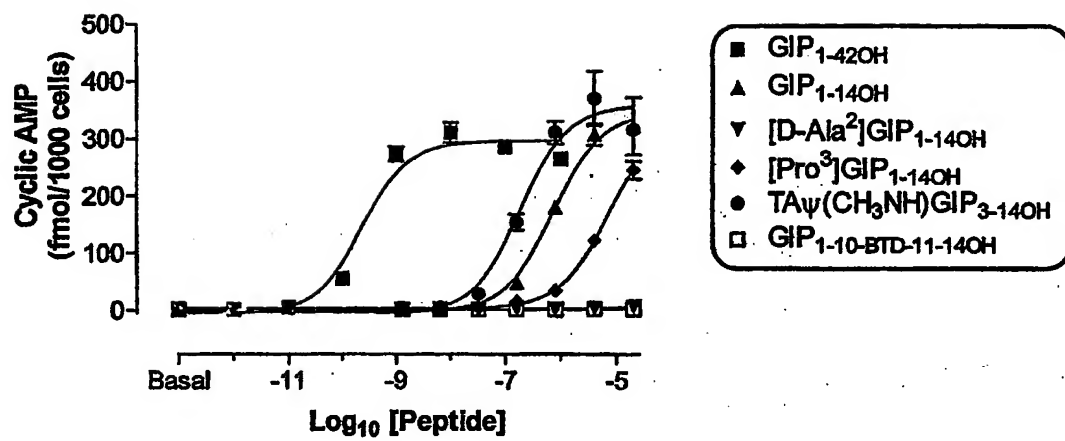
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Figure 1



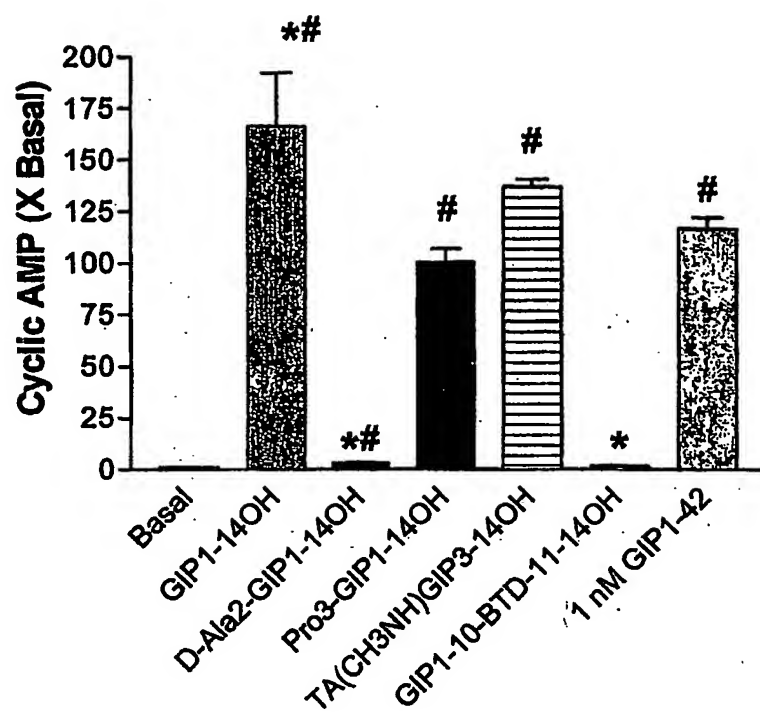
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Figure 2



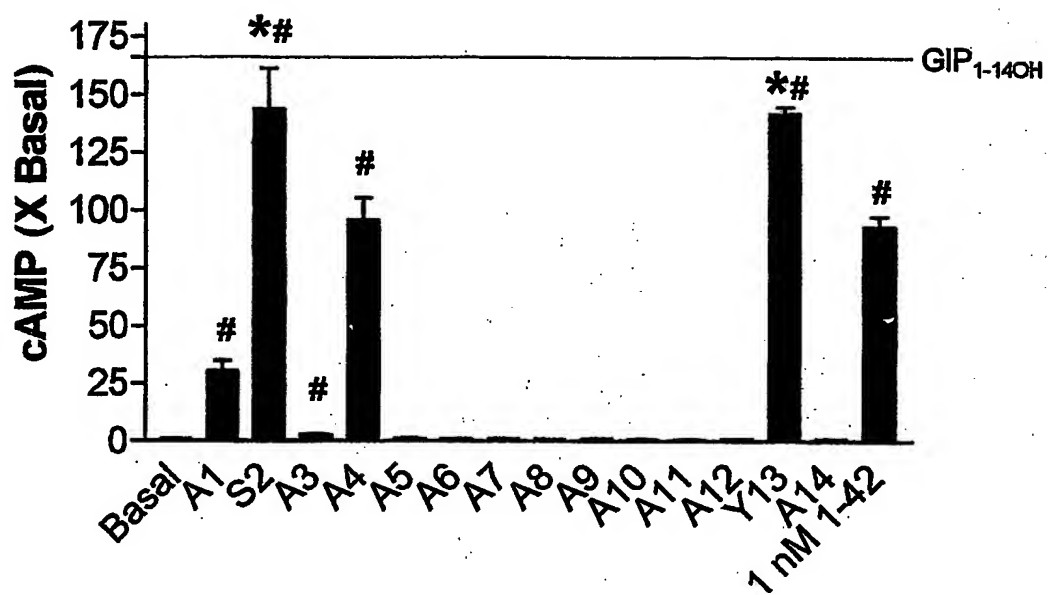
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Figure 3



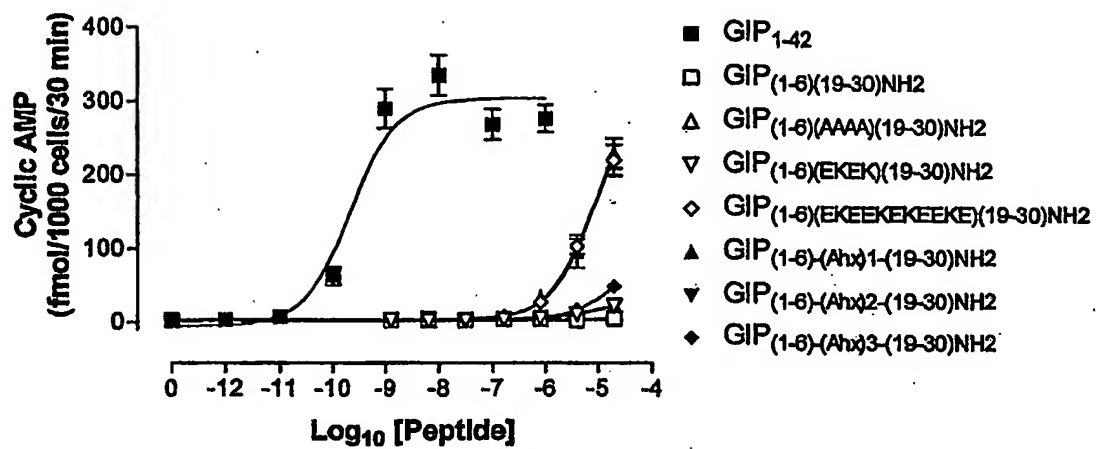
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Figure 4



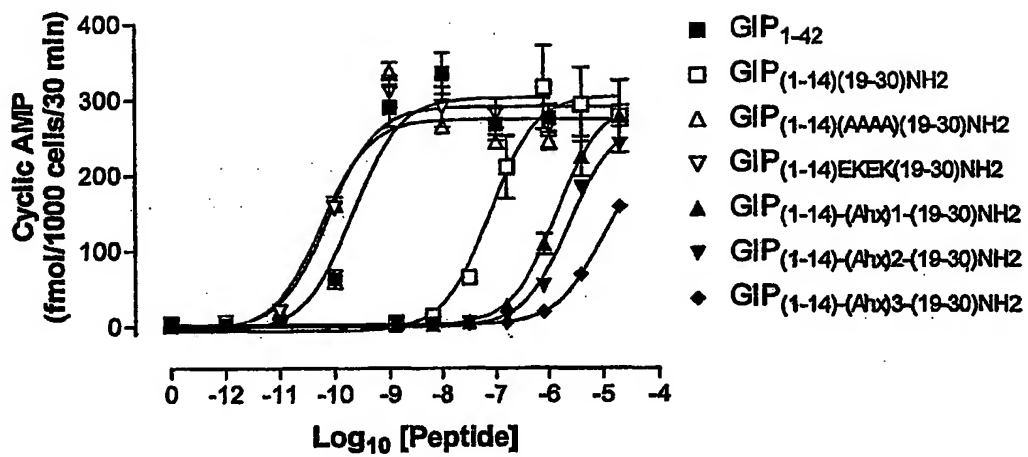
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Figure 5



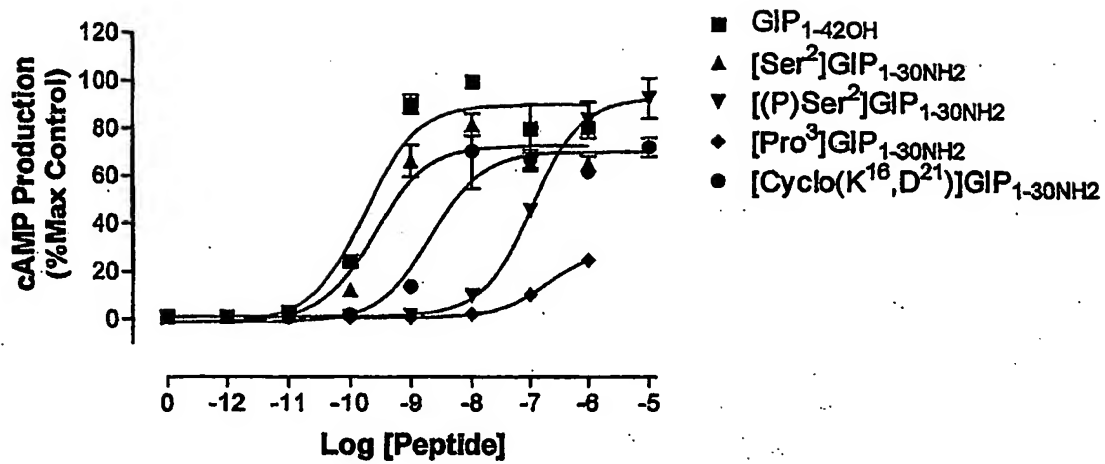
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Figure 6



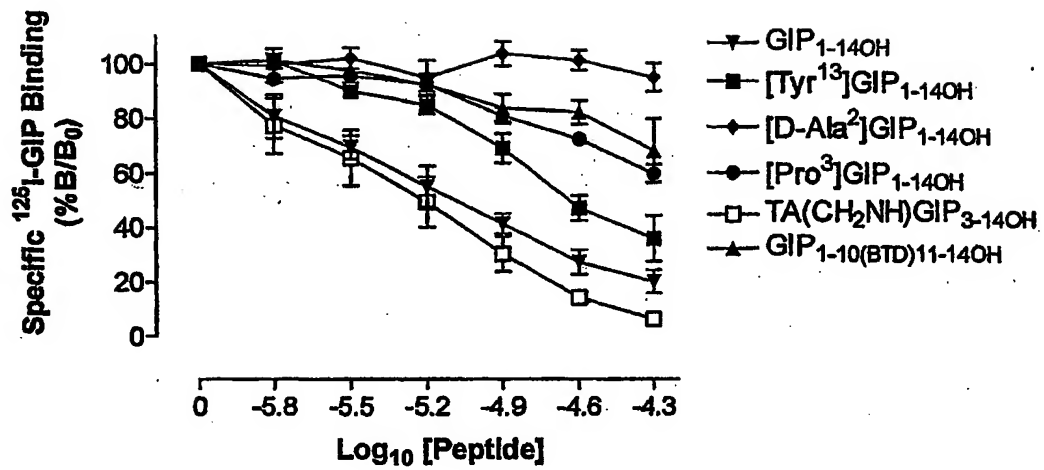
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Figure 7



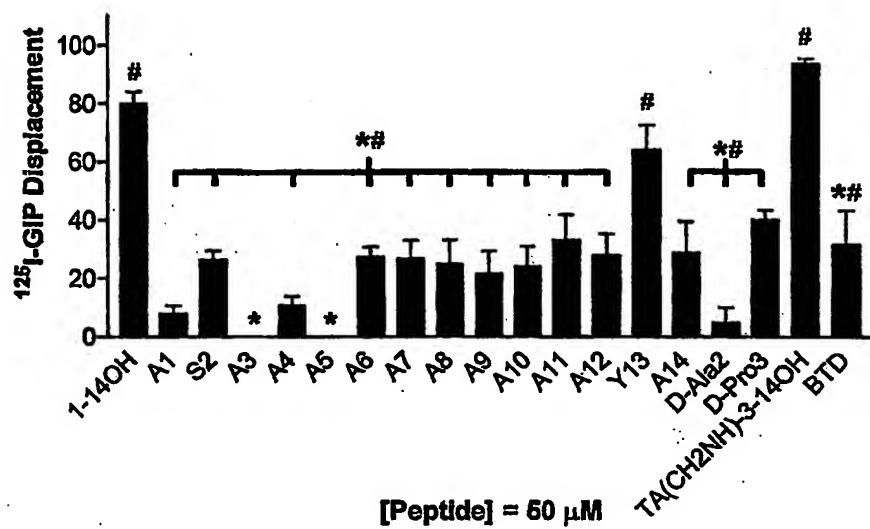
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Figure 8



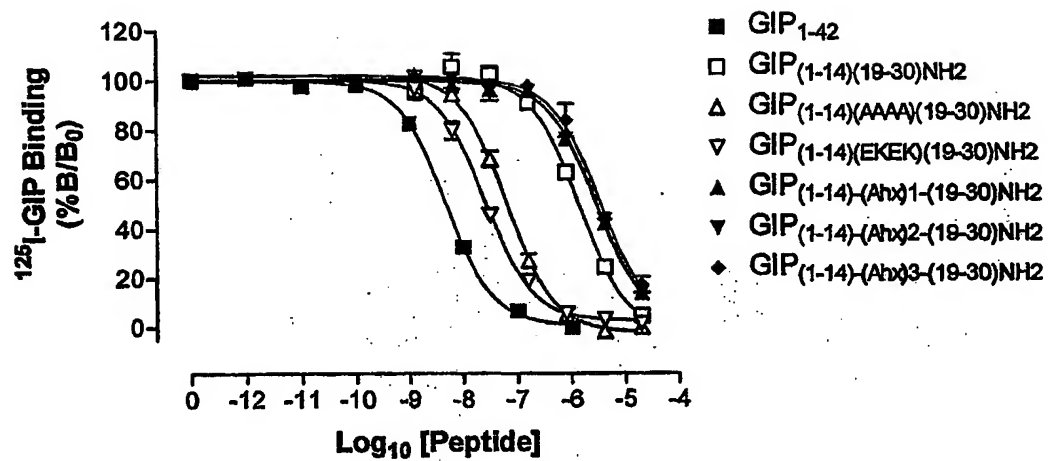
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Figure 9



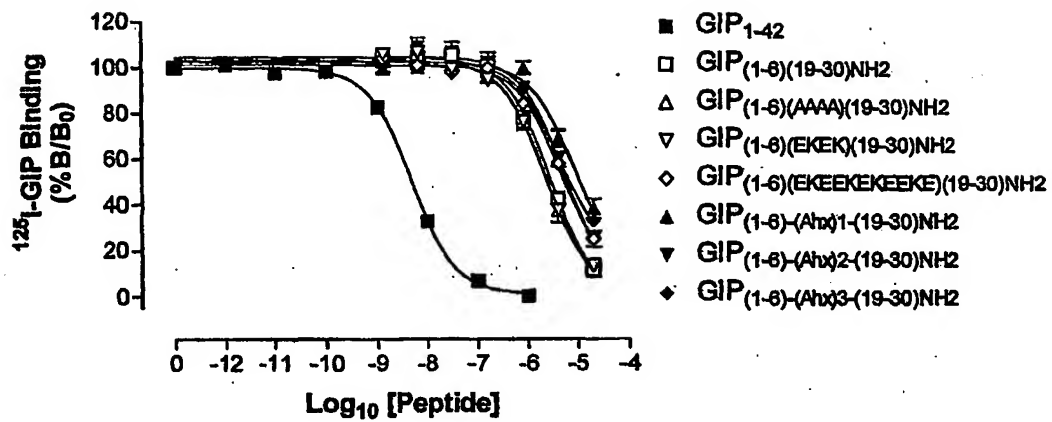
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Figure 10



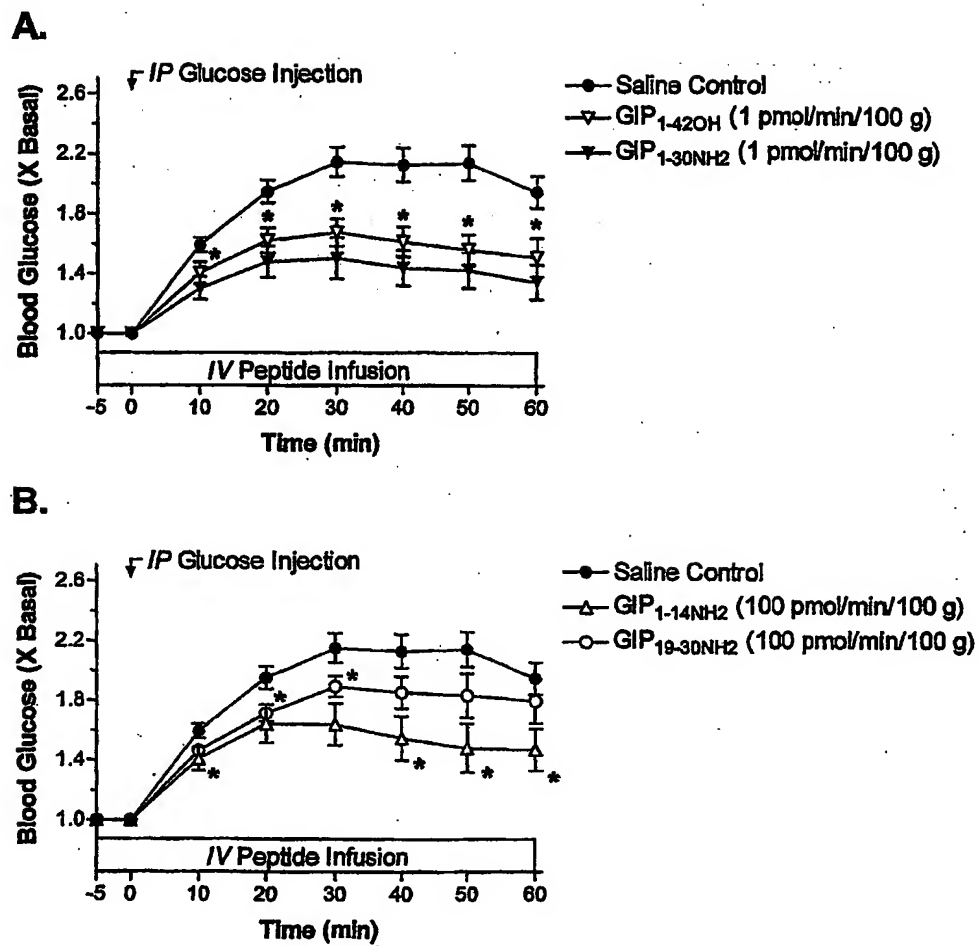
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Figure 11



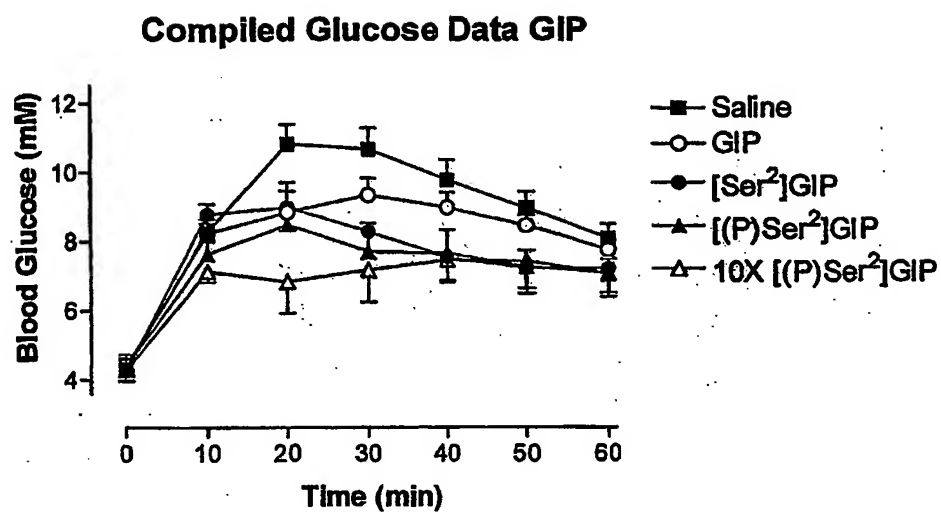
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Figure 12



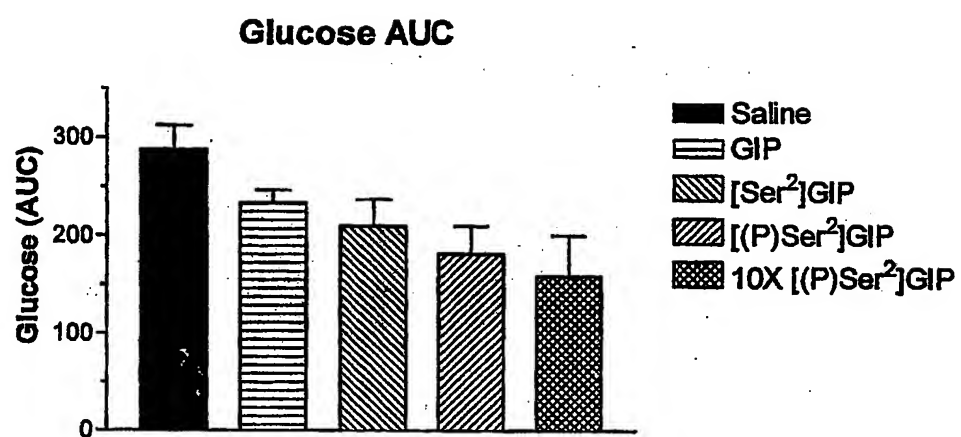
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Figure 13



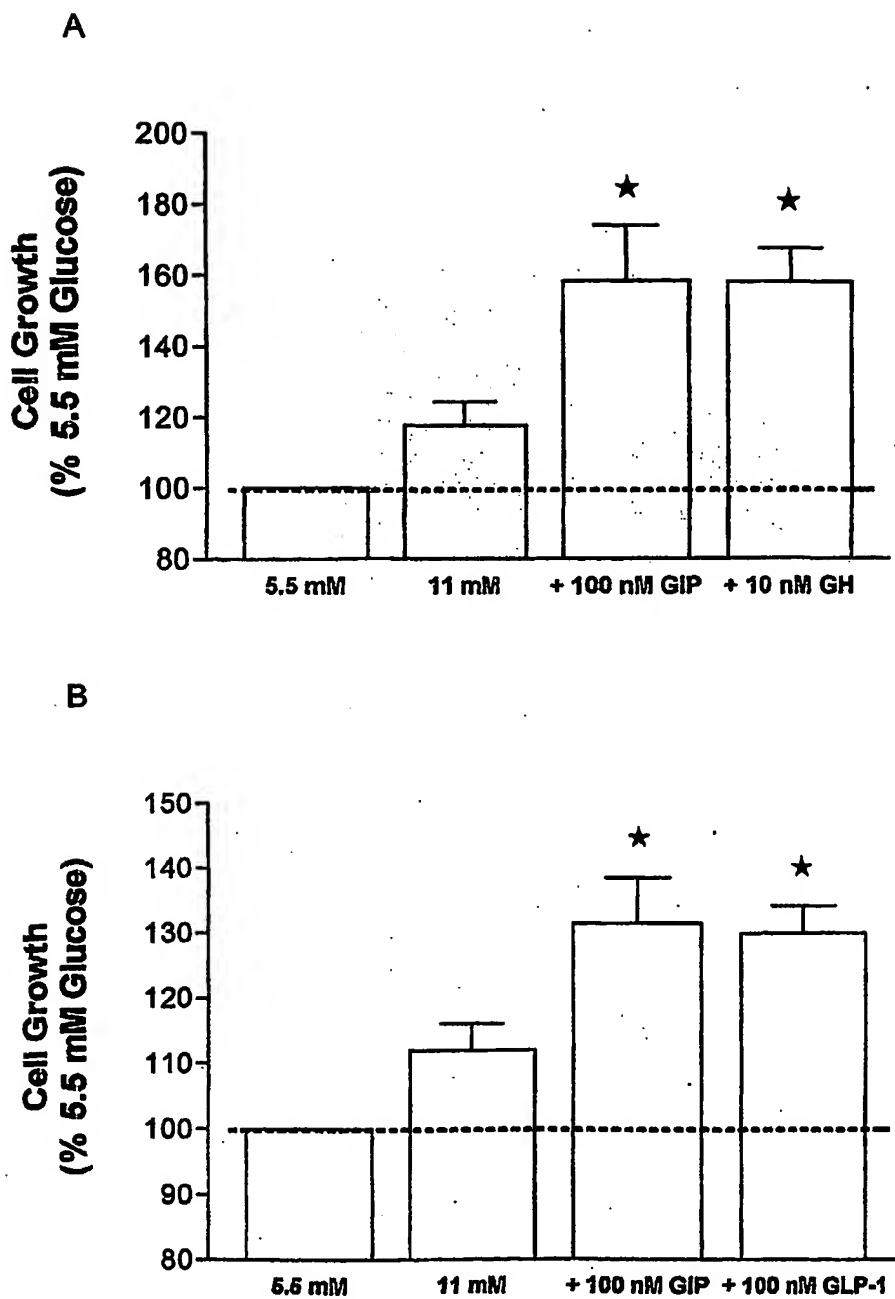
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Figure 14



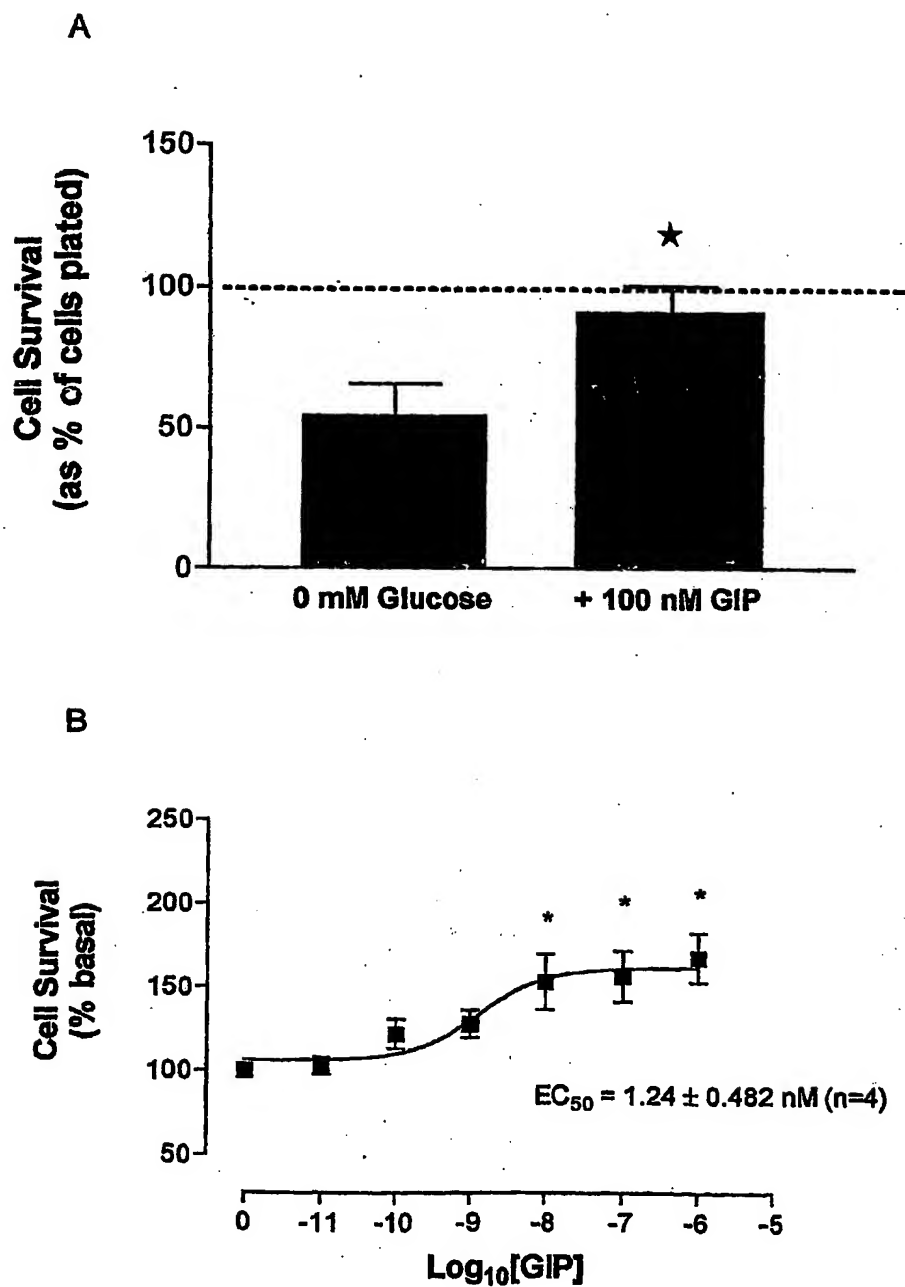
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Figure 15



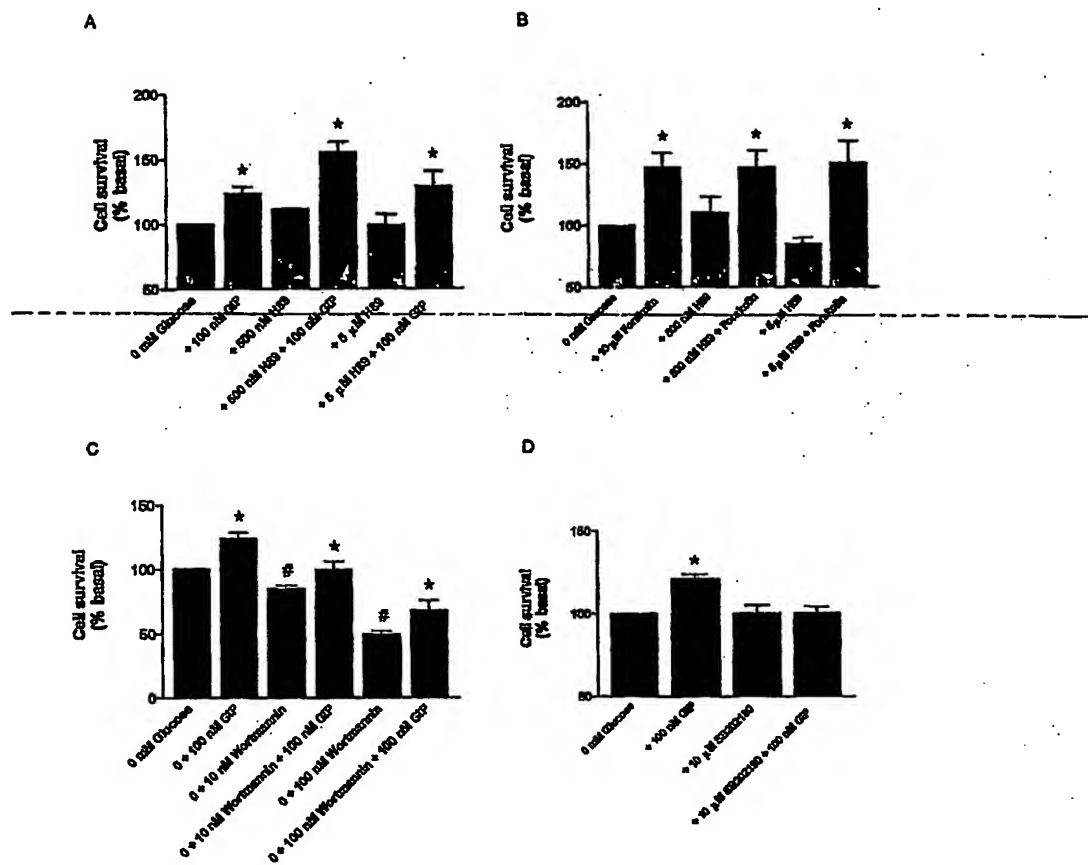
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Figure 16



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Figure 17



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Figure 18

